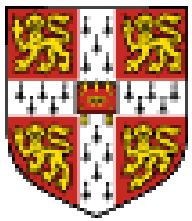
Sex Chromosome Structure and Function Part II 2015/16



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Lecture 2: Functional specialisation

- X chromosome
  - *Selective pressures*
  - *Functional categories of X genes*
  - *Disease relevance (human and model systems)*
- Y chromosome
  - *Selective pressures*
  - *Functional categories of Y genes*
  - *Candidate disease genes (Turner syndrome, male fertility)*
- Structure/function relationships
  - *Types of sequence amplification on the sex chromosomes*
  - *Consequences of repeat structures for pathogenic deletion*
  - *Transcriptional dynamics of X and Y during spermatogenesis*

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Selective pressures on X chromosome genes

X chromosomes have two features that distinguish them from ordinary autosomes.

1) 2/3 of all X chromosomes are found in females, as compared to 1/2 of all autosomes
Accelerated selection for female-benefit genes

2) The X is hemizygous in males, and functionally hemizygous on a per-cell basis in females
Recessive alleles are uncovered more readily for X chromosomes than autosomes, leading to more selection against recessive deleterious mutations and for beneficical recessive mutations

X chromosomes are enriched for male-benefit genes due to the hemizygous uncovering, but also accumulate genes for *any* highly-selected function, e.g. intelligence. A high proportion of X chromosome deletions lead to mental retardation, indicating that the X is enriched for genes with roles in brain function.

The X chromosome and disease

- Large, comparatively gene-rich
- Characteristic inheritance pattern associated with X-linked transmission
- Mapping and identification of X-linked disease genes can be achieved using standard forward and reverse genetics techniques
- Prevalence of X-linked mental retardation (+autism?)
- X chromosome link to ovarian insufficiency

The X chromosome and disease

X-linked mental retardation (XLMR)

Mental retardation affects 1-3% of the population

In males, around 10-12% of this is due to X-linked disorders,
i.e. ~1/1000 males affected

Polygenic with over 200 separate conditions identified

	Total	***Cloned***	***Mapped***
Syndromic	98	38	31
Neuromuscular	51	28	16
Nonspecific/MRX	66	16	50
Total conditions	215	82	97

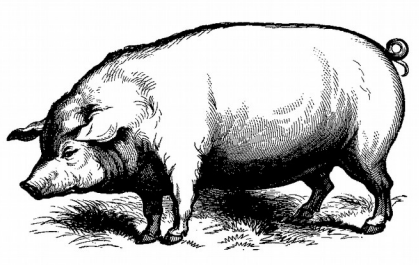
Most common: Fragile X

Fmr1 gene

~20% of XLMR cases

Trinucleotide repeat disorder

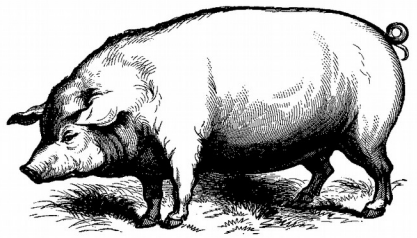
~10% of genes on the X chromosome implicated
(around double the proportion of autosomal genes)



Porcine maternal infanticide

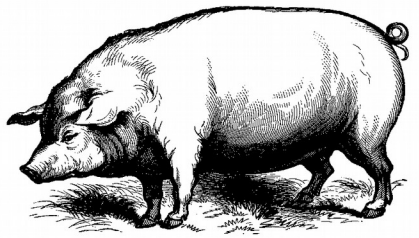
- Aggressive infanticide is characterised by pigs killing their offspring by biting them to death - usually occurs in the first 24 hrs
- Aggressive behaviour common 8-12%
- Sows prone to aggression may be restless
- Current treatment use of sedatives

Maternal Aggression as a mod for a postnatal psychosis



- Childbirth is a period of substantial rapid biological and psychological change which leads to various postnatal conditions in humans:
  - **‘BABY BLUES’** - 50% of women. Onset - 3-5days. Symptoms - crying and depression, which only last for a few days.
  - **POST-NATAL DEPRESSION** - 5-15% of women. Can last for a few months if untreated. Symptoms - poor sleep and appetite, suicidal thoughts and self-blame (Jones et al., 2001).
  - **PUERPERAL PSYCHOSIS** - 1 in 1000 births – Onset within the first month. Symptoms - rapid fluctuation of mood (manic and depressive symptoms), confusion and perplexity, in addition to symptoms of psychosis (delusions, hallucinations, marked behavioural disturbance). Thoughts of self-harm may be due to feelings of guilt, self-worthlessness or hopelessness (Cantwell and Cox, 2003).

Maternal Aggression as a mod for a human condition



- ***Puerperal Psychosis***

- affects 1/1000 deliveries

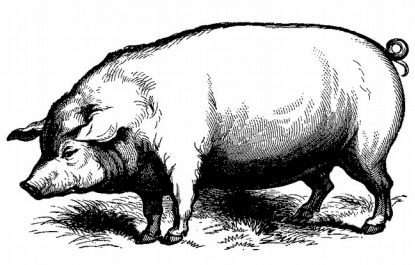
- genetic and environmental components
  - hormone levels important
  - pregnant women with pre-existing bipolar disorder (also known as manic depression) have an increased risk of puerperal psychosis.
  - siblings at increased risk
  - see mother-daughter cases
- highest risk at first pregnancy- no obvious disturbance during
- triggers include restlessness and lack of sleep

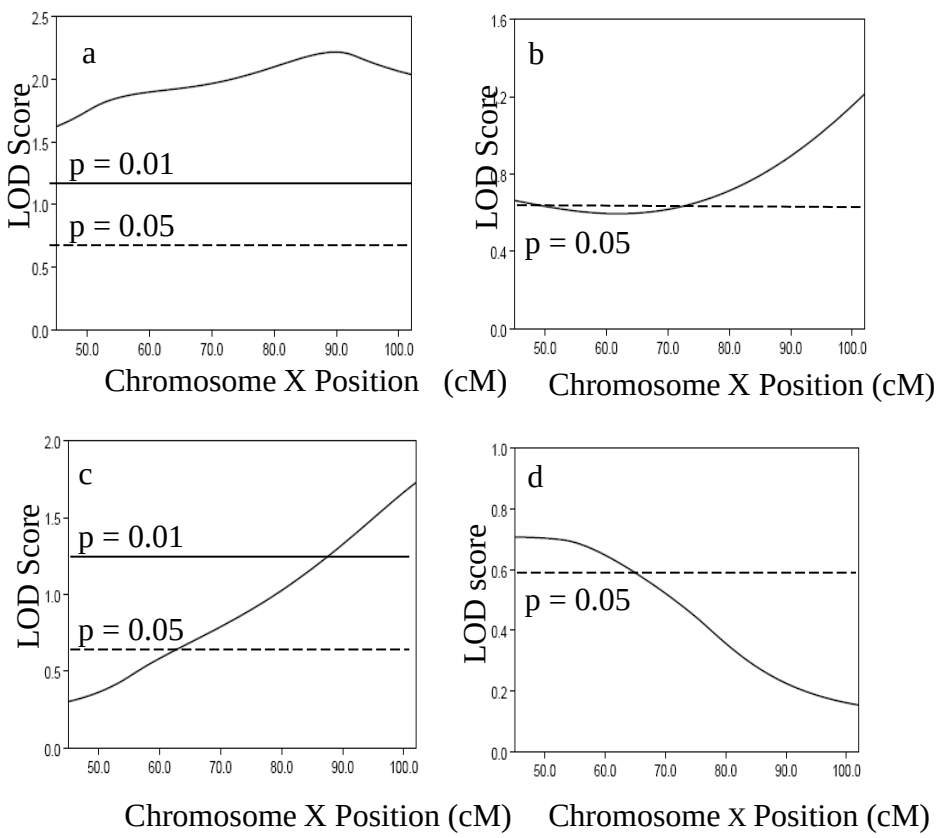
- ***Aggression in pigs***

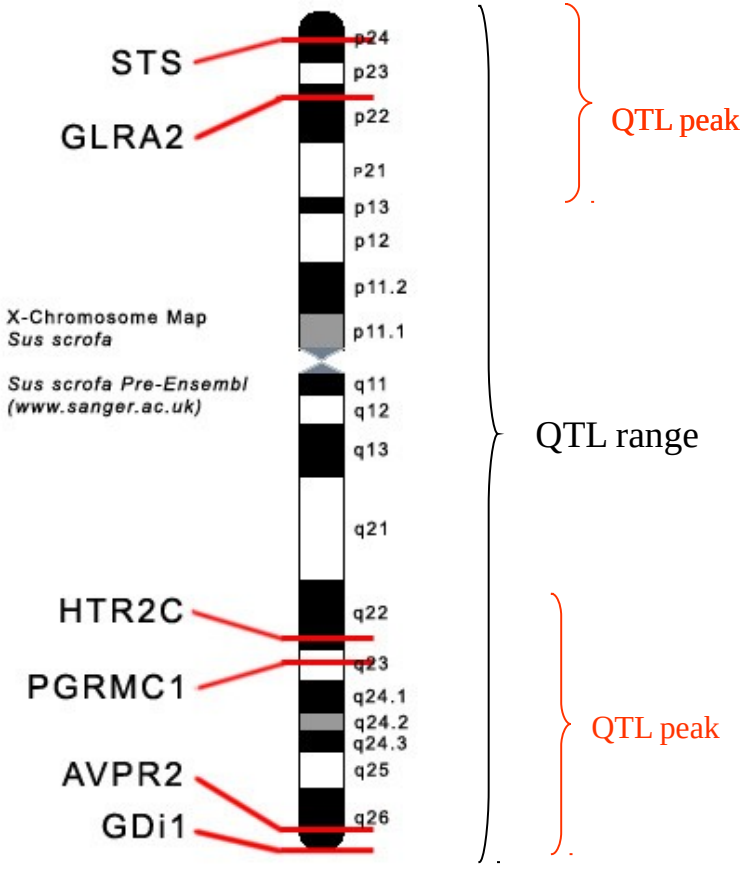
- affects approx. 10% of animals in commercial herds (breed dependent)
- genetic and environmental components
  - hormone levels thought important
  - siblings at increased risk
  - dam – daughter cases
  - breed dependant

- highest risk at first pregnancy (gilts) limited indication during
- behavioural patterns show animals are restless, lack of passivity expected with normal mothering

SSCX

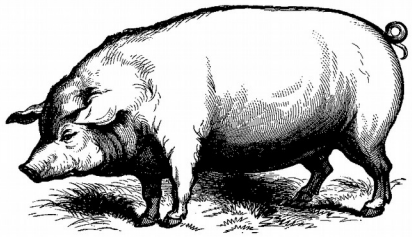






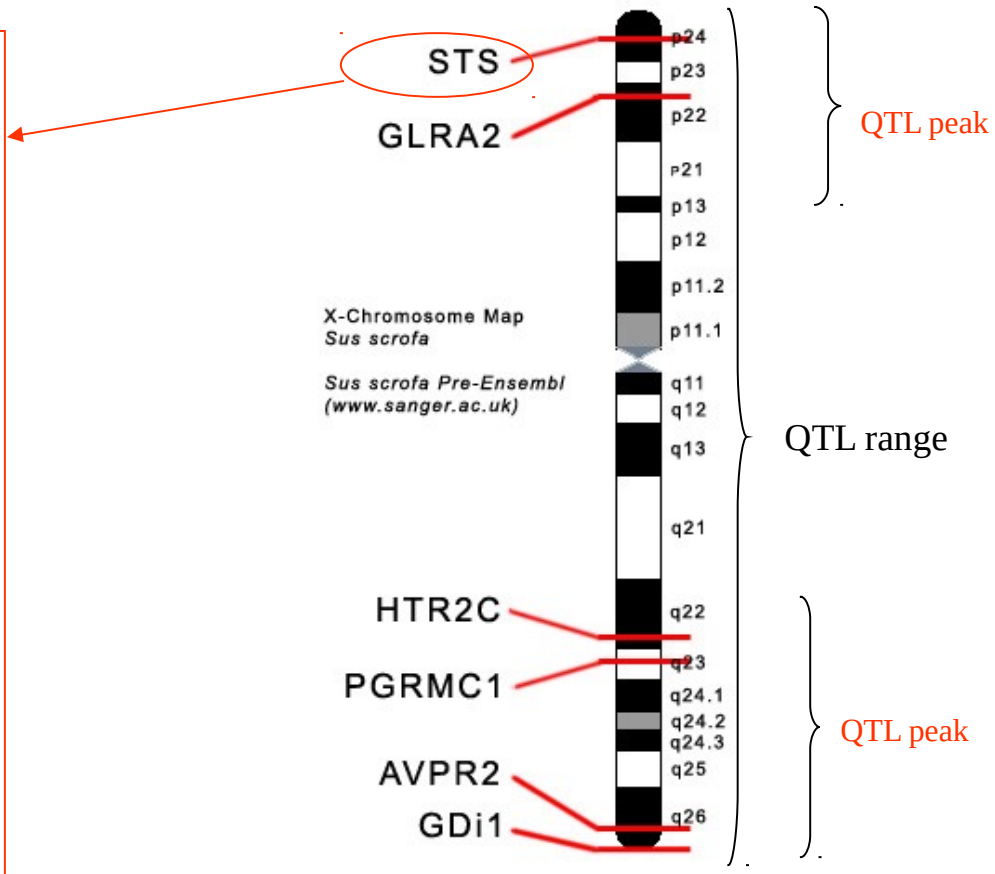
SSCX QTL scans **(a)** All lines, **(b)** Large white, **(c)** Landrace/Duroc, **(d)** All other lines combined

SSCX



Steroid Sulphatase

- Catalyses the conversion of sulphated steroid precursors to oestrogens during pregnancy
- Involved in the biochemical pathways of neurosteroids with interactions with neurotransmitters
- Inhibition of STS has been shown to increase aggression in mice
- SNPs associated with aggression



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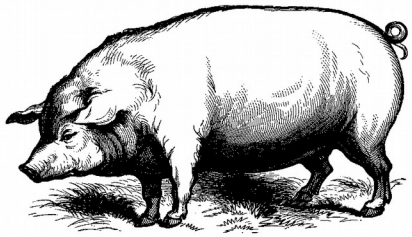
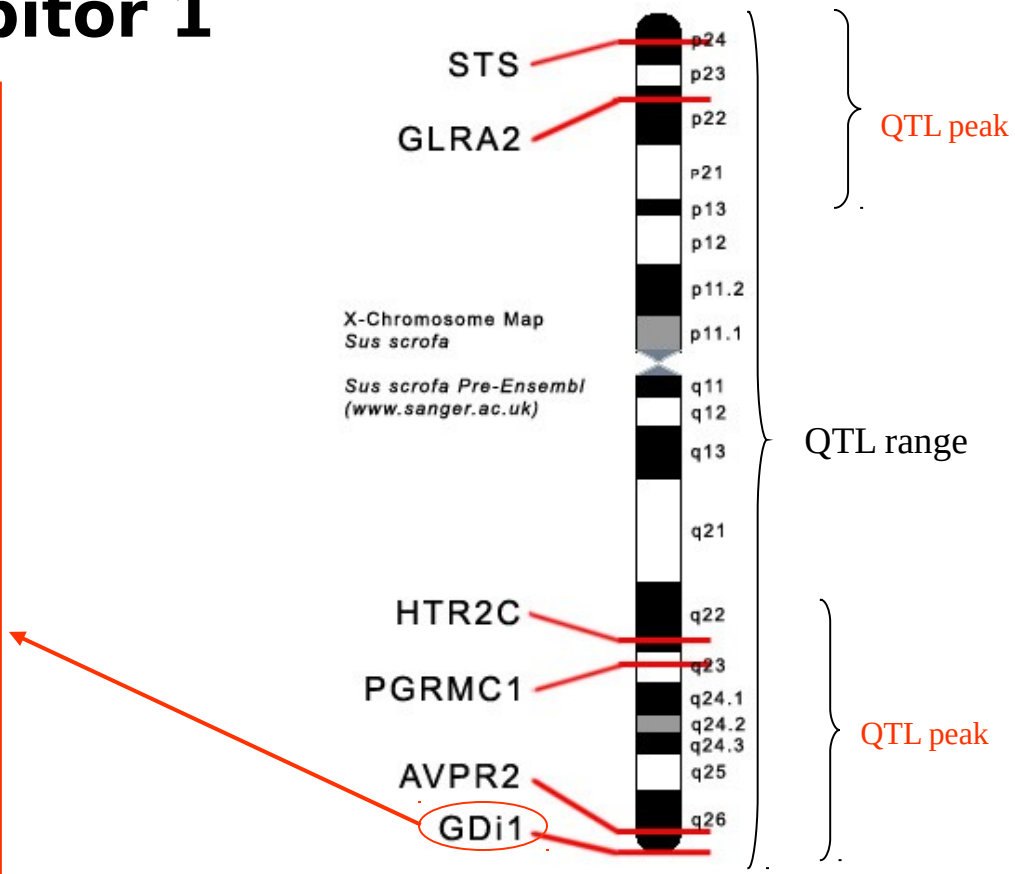


GDP Dissociation Inhibitor 1

- Loss of function and null mutations in the GDi1 gene have been shown to be responsible for X-linked non-specific mental retardation in humans
- GDi1 knockout mice were found to have impaired associative memory as well as altered social behaviour
- SNPS associated with aggression



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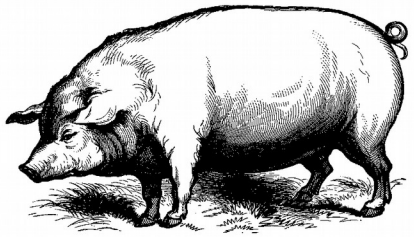
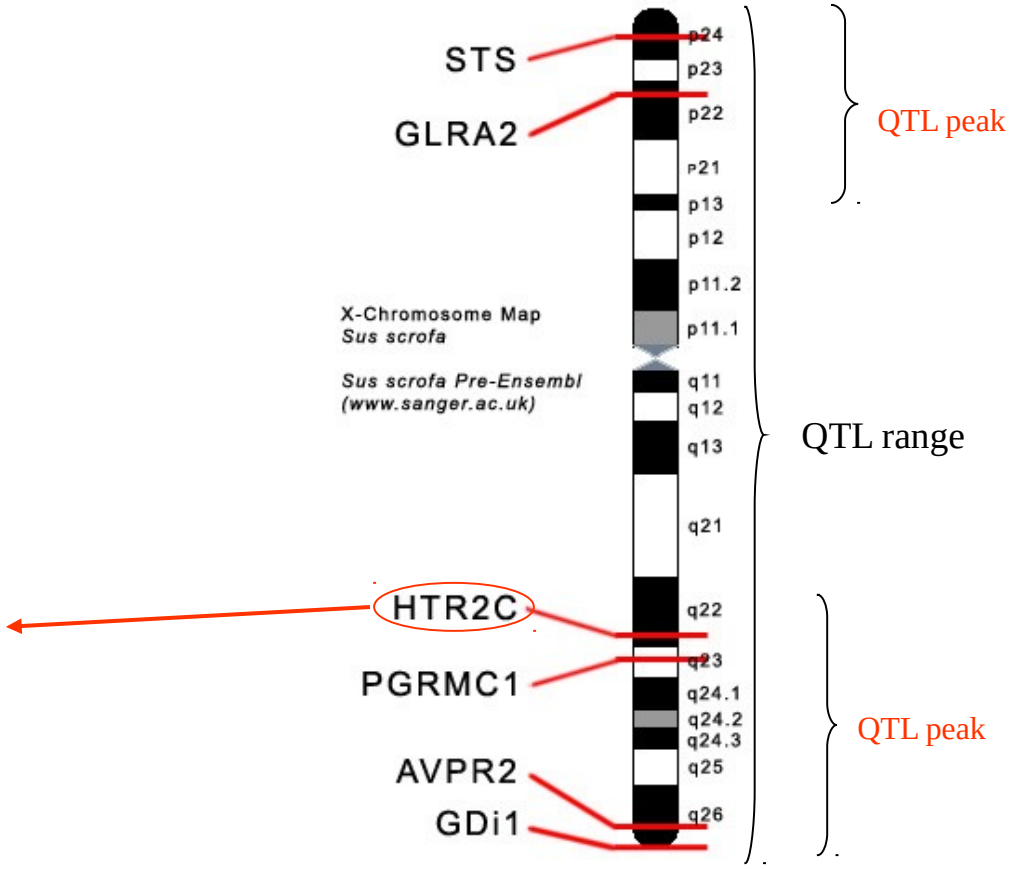


Serotonin receptor

- Overactivity linked to suicide, depression and anxiety in humans
- Human polymorphisms linked to a range of phenotypes such as depression, OCD, anxiety.
- SSRIs a common class of antidepressant drug

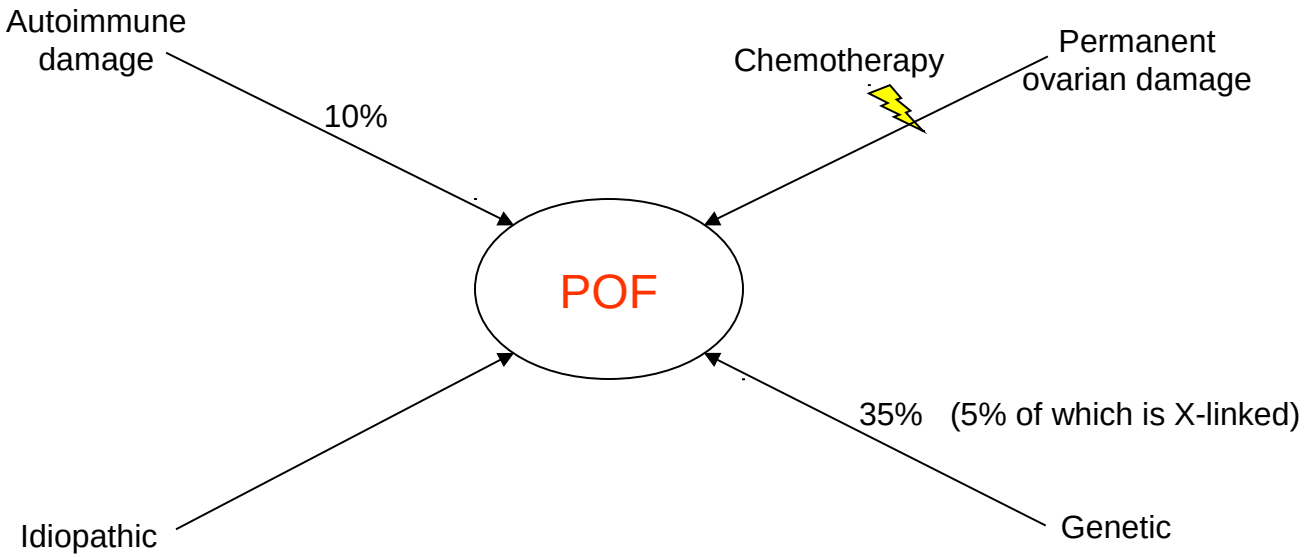


The X chromosome and disease

Premature Ovarian Failure (POF) – early menopause

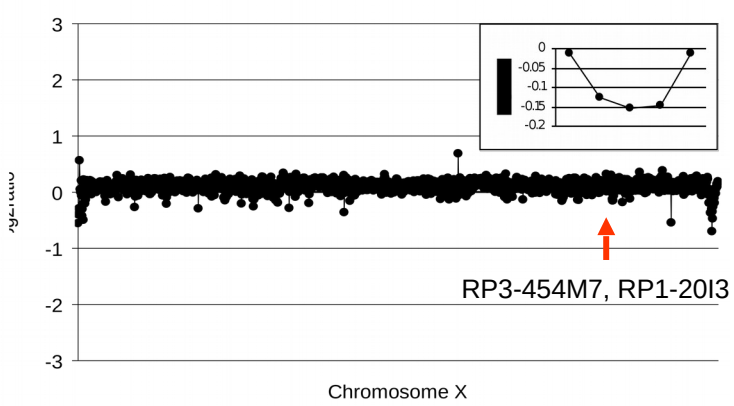
Defined as amenorrhoea for > six months before the age of 40, with an FSH serum level > than 40 mIU/ml. May involve:

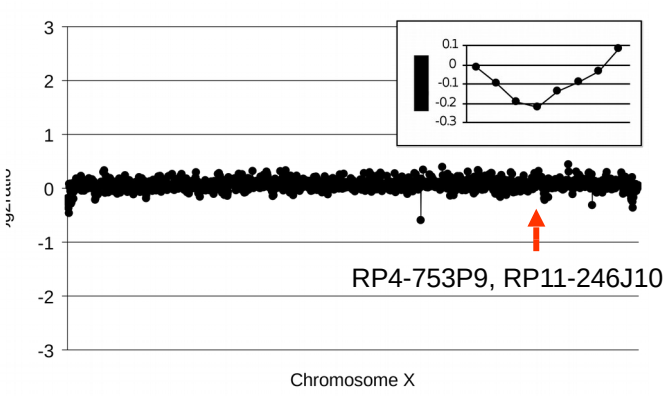
- Decreased follicular formation
- Increased follicle loss
- Unresponsiveness of follicles to hormone stimulation.



Sex chromosomes and disease

Premature Ovarian Failure (POF) – early menopause





Array CGH used to compare DNA from 40 POF patients to normal female control DNA. 50 CNV regions were found in 31 patients, of which 26 regions (21 patients) remained after excluding known common polymorphisms.

Candidate gene analysis is ongoing

Sex chromosomes and disease

Premature Ovarian Failure (POF) – candidate genes

GENE	Function/ Potential involvement in POF	Expressed in Ovary	Escapes X inactivation
ZFX	Homozygous mutant XX mice shortage of oocytes resulting in diminished fertility and shortened reproductive lifespan (Luoh et al 1997)	YES	YES
CENPI	The product of this gene is involved in the response of gonadal tissues to follicle-stimulating hormone (Slegtenhorst-Eegdeman et al 1995)	NO	NO
BCORL1	Deletion may lead to insufficient repression of apoptosis resulting in atresia of ovarian follicles (Pagen et al 2007)	YES	NO
TSPAN7	The product of this gene mediate signal transduction events	YES	NO
USP9X	Suggestive association with POF in GWA study (Knauff et al 2009)	YES	YES
VCX	Encodes small protein of unknown function, expressed in male germ cells only	NO	NO
XNPEP2	Breakpoints disrupting gene reported in POF patients (Pruiett et al 2000, Mumm et al 2001)	NO	PARTIALLY

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  - *Transcriptional dynamics of X and Y during spermatogenesis*

Selective pressures on Y chromosome genes

Y chromosomes (excluding PAR) have three key factors that distinguish them from autosomes:

1) All Y chromosomes are found in males
   Accelerated selection for male-benefit genes

2) All Y chromosomes are hemizygous
   There is no such thing as a recessive Y-specific gene – all mutations in such genes are open to selection immediately

3) The Y chromosome does not undergo interchromosomal recombination
   Leads to degeneration as discussed in previous lecture

In general, the third of these is the most important factor, however as discussed previously, male-benefit genes and some housekeeping genes escape degeneration

The Y chromosome and disease

- Small, gene-poor
- Non-recombining nature means that standard genetic mapping techniques cannot be used – need to construct a deletion map based on

  deletion/rearrangement breakpoints rather than a map based on recombination events.
- Not necessary for life – females manage fine without one!

What can we conclude about Y chromosome gene function and how?

- Two categories of gene on Y: housekeeping genes that have not lost their function and whose homologues escape X inactivation, and specific male-benefit genes such as spermatogenic factors.

Will examine two categories of phenotype:

1. Regions involved in the Turner phenotype

2. Regions involved in spermatogenesis and infertility

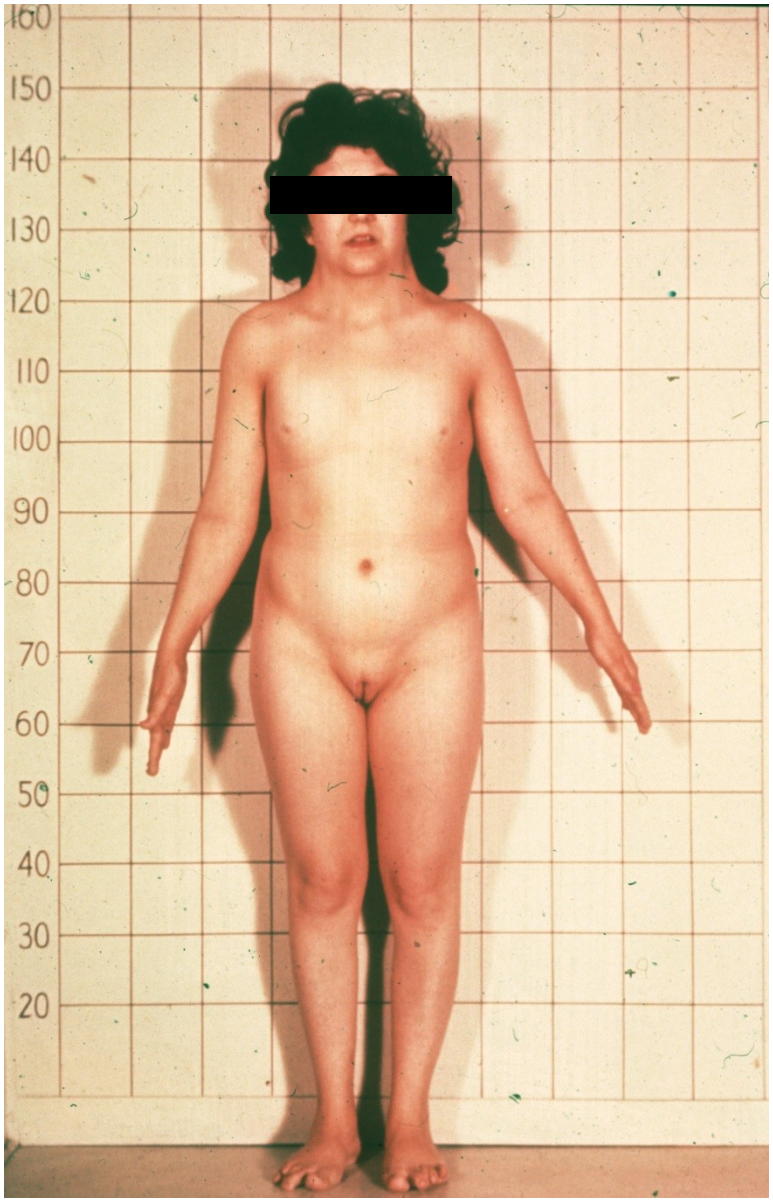
Turner Syndrome - 46,X0 - single X chromosome

Gonadal anomalies:

- streak gonads through early degeneration of the ovary - infertile

Somatic anomalies:

- short stature
- skeletal anomalies e.g. wide carrying angle
- poor lymphatic system development (lymphatic hypoplasia). Leads to oedema during development and consequent webbing of the neck. Puffy feet in new-born
- Heart defects. Coarctation of the aorta
- Horse-shoe kidney
- Poor secondary sexual differentiation



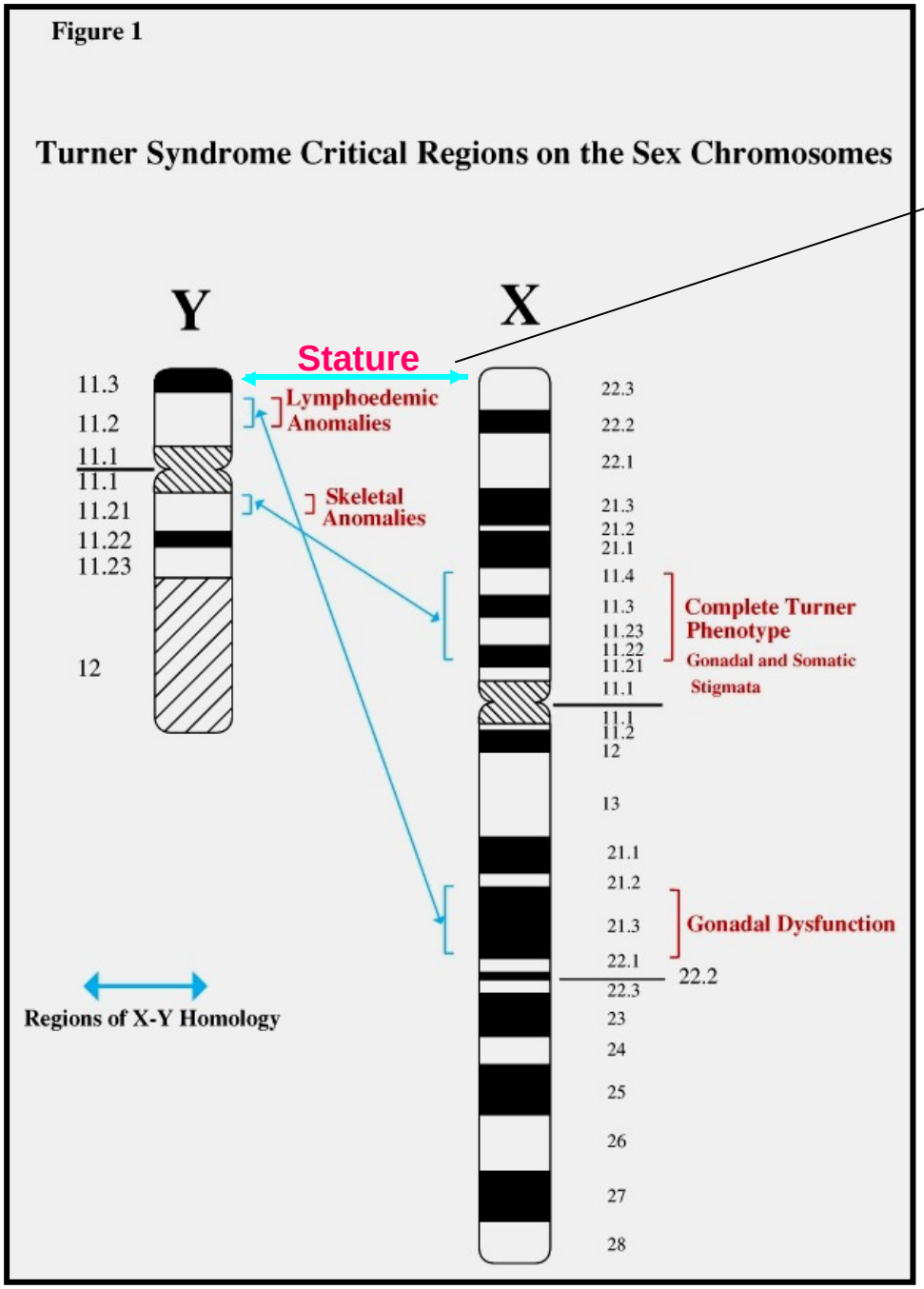
Defining Turner syndrome genes

Non-ovarian symptoms (e.g. stature, lymphoedema, skeletal abnormalities)

- Gene function conserved X-Y since either X or Y is protective
  Likely to be either a PAR gene or a recent addition to the non-recombining region of the X/Y.
- X copy escapes X inactivation, since a single X is insufficient
  Again suggests PAR or recent addition
- Can be mapped by analysing X deletions or Y deletions, together with sequence analysis of regions of X/Y homology.

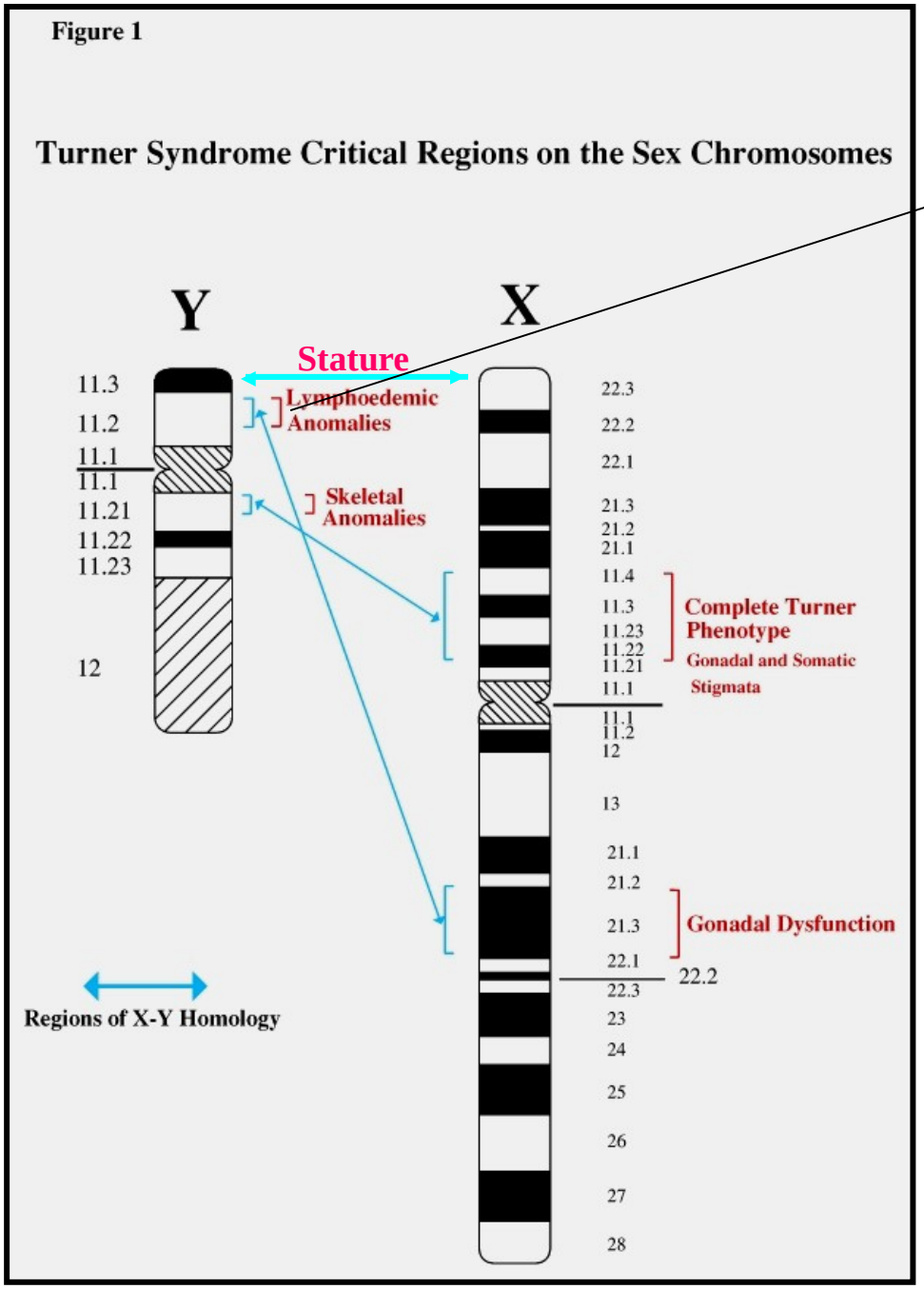
Ovarian symptoms – streak gonad, premature ovarian failure

- Cannot conclude whether there is a Y equivalent. Can conclude that at least some of the X gene(s) responsible likely escape X inactivation.



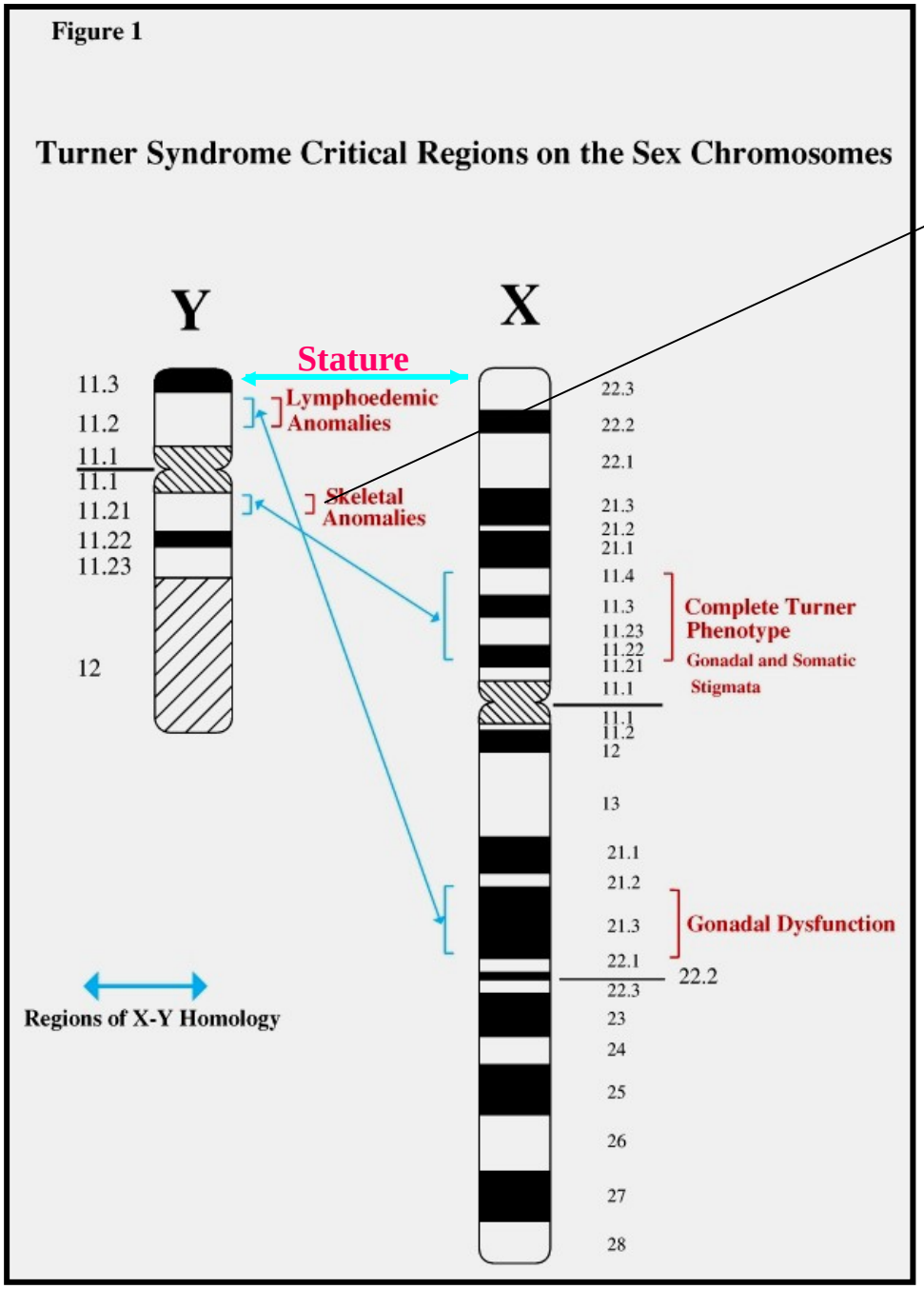
SHOX gene in PAR1

- **homeobox gene with mutations found in idiopathic short stature**
- **Haploinsufficiency of gene product in Turner patients**
- **Phosphorylation-sensitive function of SHOX is directly involved in chondrocyte differentiation and maturation**



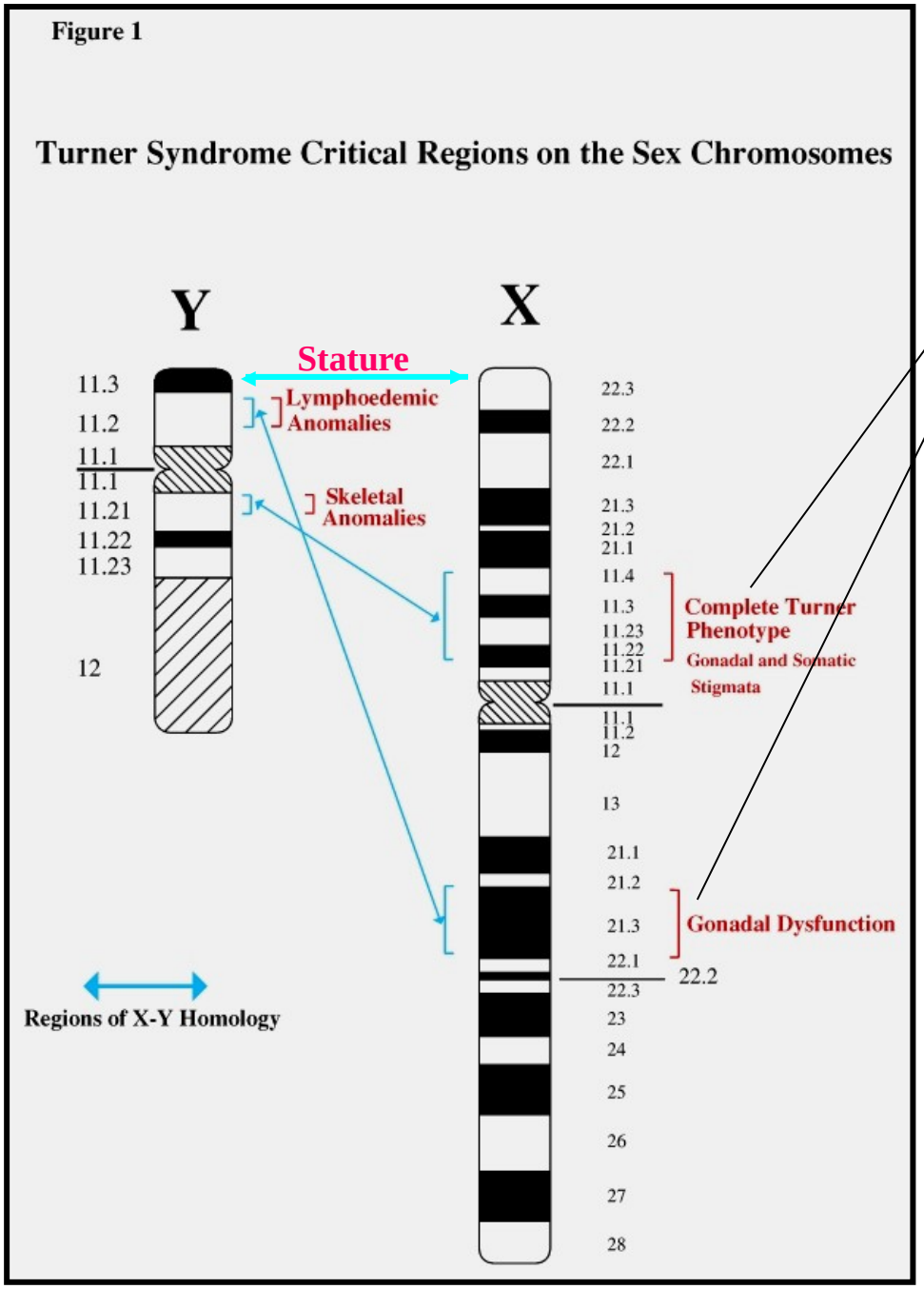
Lymphoedema locus

- **Mapping narrowed down to the recent human-specific X-Y transposition**
- **Candidate genes in region are** *Tgif2ly* and *Pcdh11y*



Skeletal growth locus

- **Mapping narrowed down to near Y centromere**
- **X location in Xp**
- **No candidate gene**



Ovarian phenotype

- **Mapping on Xp (as expected from the fact it escapes X inactivation) and on Xq**
- **No clear candidates**
- **Gene rich nature of X and reduced fertility means isolating one or more candidates will be difficult**
- **One avenue for approach is to investigate fertility factors in farm animals where much more breeding data is available**

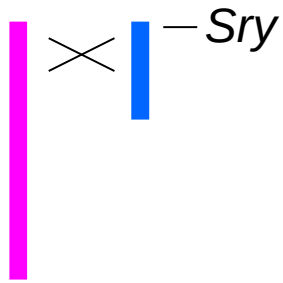
Will examine two categories of phenotype:

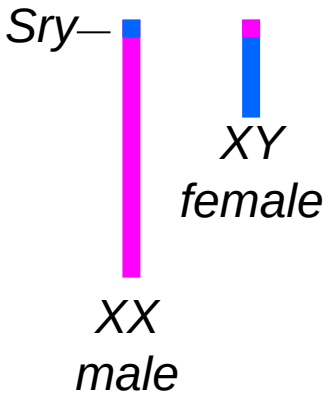
1. Regions involved in the Turner phenotype

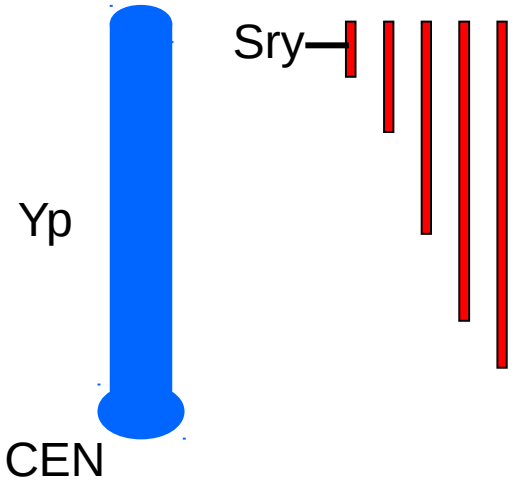
2. Regions involved in spermatogenesis and infertility

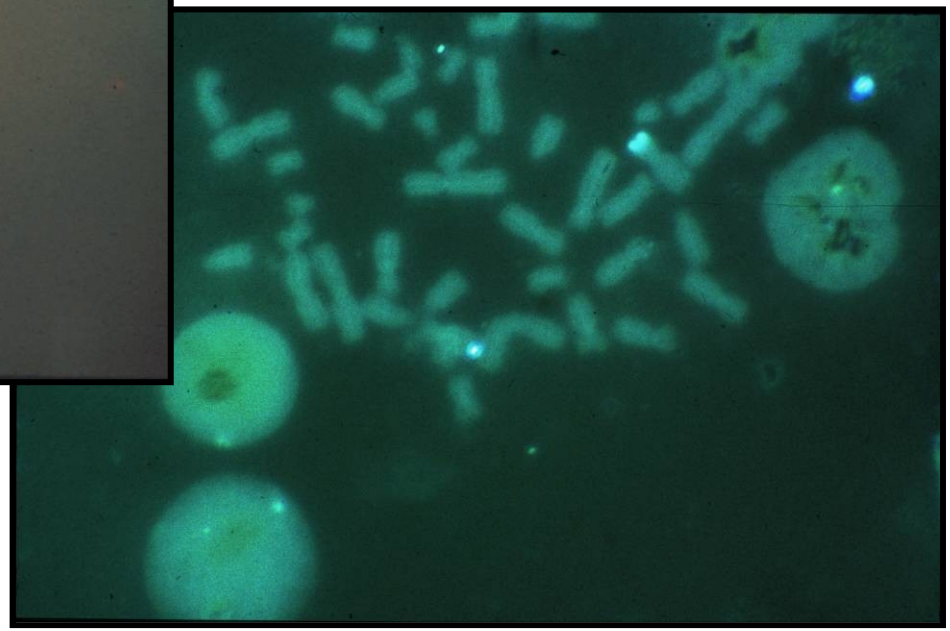
STRUCTURAL ABNORMALITIES

- Dosage effects
  e.g. sex reversal







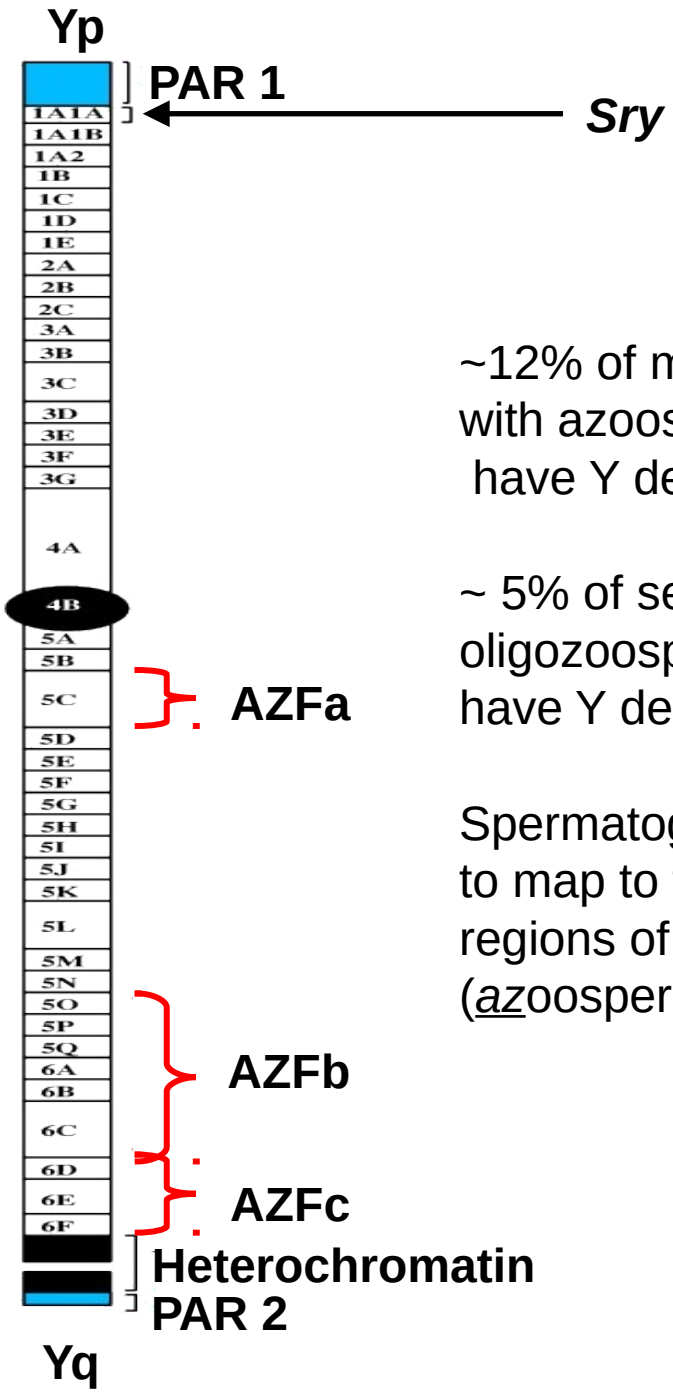


Deletion Map of the Y

Each boundary on the map represents a breakpoint from a specific patient.

Correlation of:

1. **Phenotypes to deletion intervals**
2. **Markers to deletion intervals**
3. **Overlapping YAC, PAC and BAC maps built using markers to phenotypes**
4. **Sequence organisation to interval**

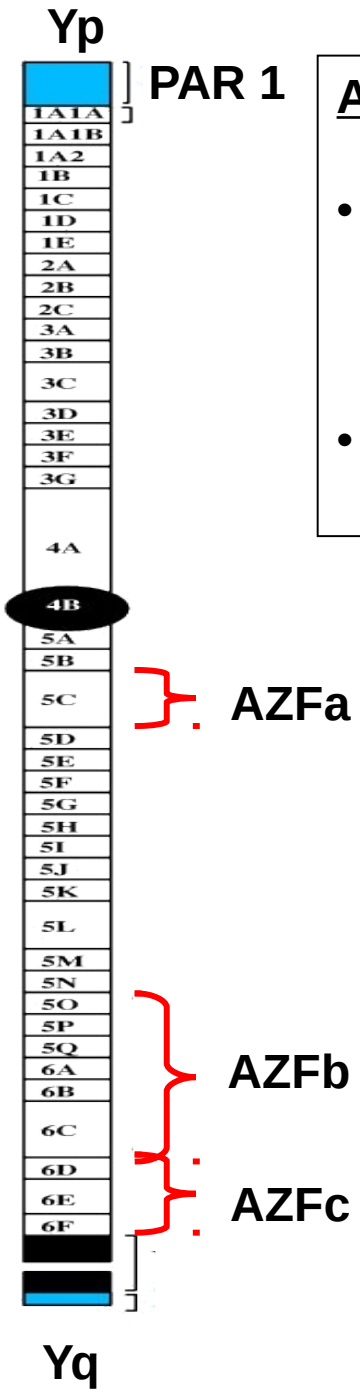


~12% of men with azoospermia have Y deletions

~ 5% of severely oligozoospermic men have Y deletions

Spermatogenic failure found to map to three separate regions of Yq, named AZF (*az*oospermia *f*actor) a, b, c.

AZF regions are associated with different phenotypes



AZFa

- Predominantly azoospermia
  - Type I Sertoli Cell Only Syndrome: no spermatogonia
- Oligozoospermia seen in smaller deletions

AZFb

- Diverse spectrum of phenotypes
- Azoospermia
  - Spermatogenic arrest rather than SCO
- Oligozoospermia seen in smaller deletions

AZFc

- Diverse spectrum of phenotypes
- Azoospermia
  - Spermatogenic arrest in some cases
  - type II SCO I others
- Oligozoospermia seen in smaller deletions

Relationship of AZF deletion to phenotype is complex:

- **At all three loci larger deletions leads to increased severity of phenotype**
  Incomplete penetrance, or involvement of multiple genes

- **Apparent diverse partial deletions of each locus associated with diverse phenotypes**
  Potential involvement of multiple genes per locus;
  ambiguity in scoring deletions

- **Apparent similar deletions in different individuals associated with different phenotypes**
  Interaction with modifying genes in genetic background;
  ambiguity in scoring deletions

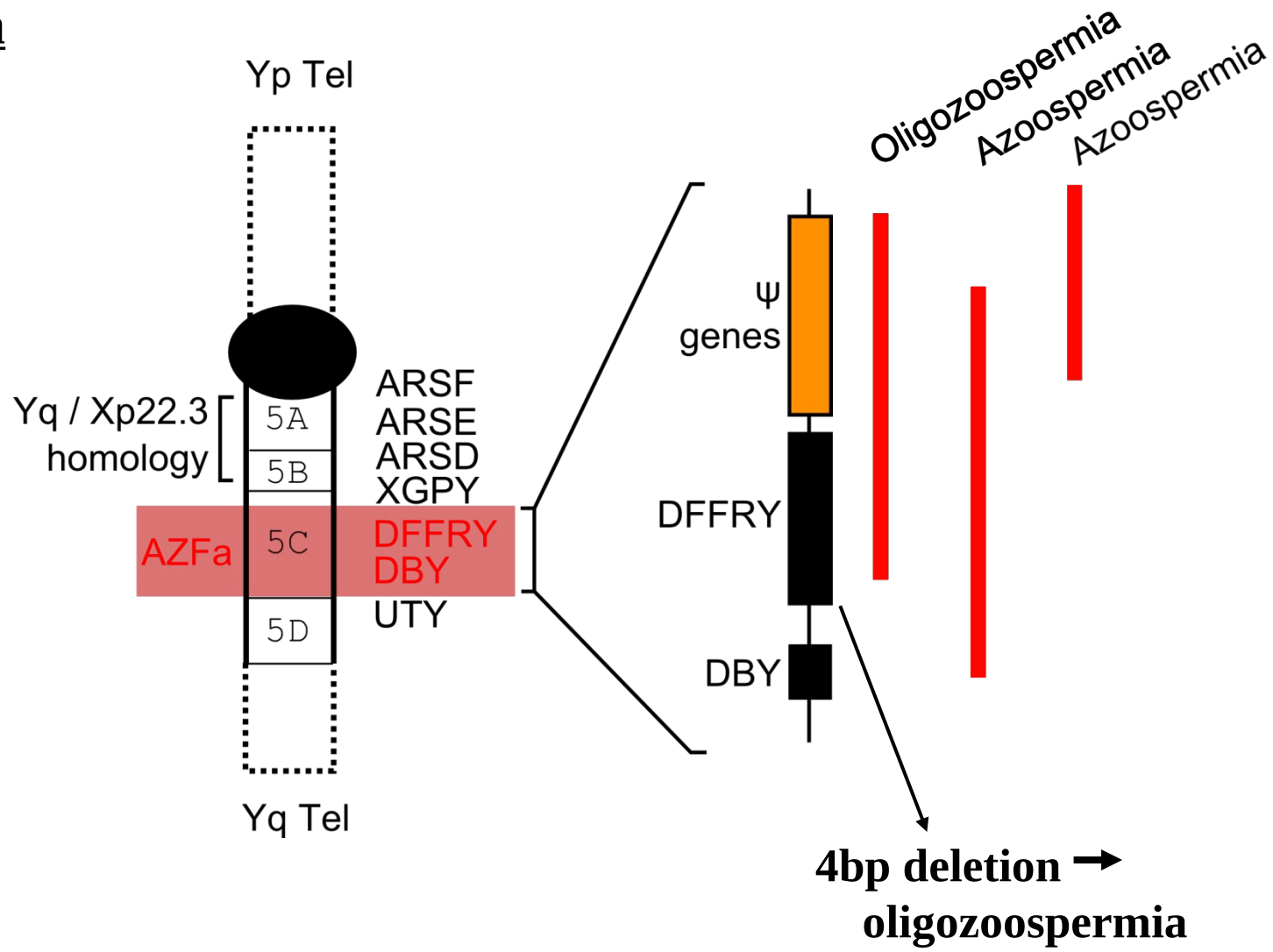
- **Transmission of deletions associated with AZFc based infertility**
  Progression to severe phenotype with age and generation
  May be a deletion expansion in unstable region of the Y?
  Mosaicism for intact Y chromosome modifying phenotype

What candidate genes reside in AZF deletion intervals?

Problematic providing direct genetic evidence linking gene to phenotype

- **Predominant lesions are deletions - difficult to assess contribution of different genes where multiple genes involved**
- **Many of the genes are in multigene families - which member or combination of members is associated with the phenotype?**
- **Almost universal failure to find point mutations**

AZFa



DFFRY (USP9Y)

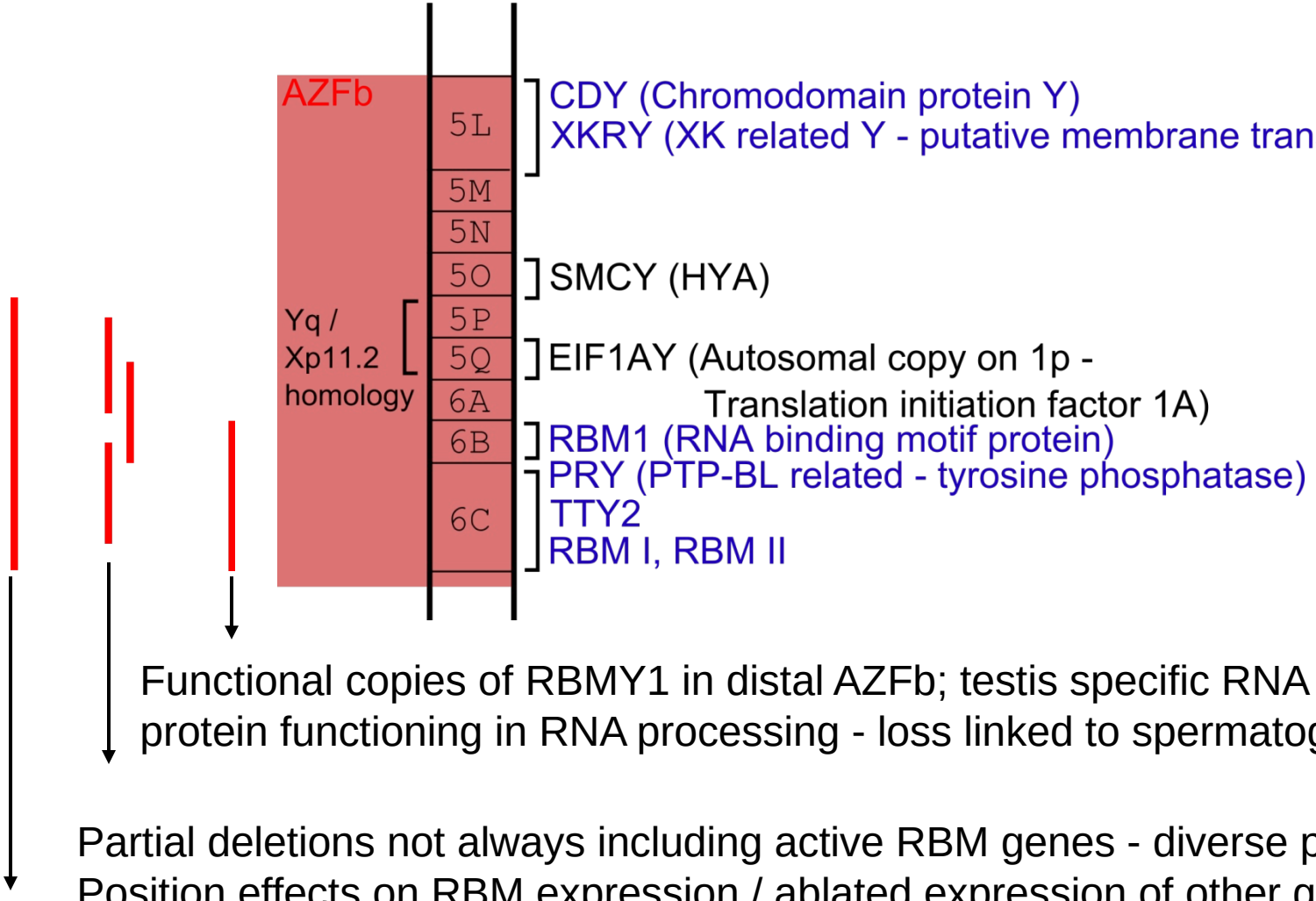
Ubiquitin specific protease - ubiquitin dependent protein degradation

***DBY* (*Ddx3y*) -** RNA helicase containing dead box motif

Position effect on expression of *DFFRY* and/or *DBY*?

AZFb

[Testis specific & part of multigene families]



Functional copies of RBMY1 in distal AZFb; testis specific RNA binding protein functioning in RNA processing - loss linked to spermatogenic arrest

Partial deletions not always including active RBM genes - diverse phenotypes
Position effects on RBM expression / ablated expression of other genes ?

Complete deletions mainly spermatogenic arrest at spermatocyte/spermatid stage

RBM Gene Family

1. X-Y homologous gene with X gene mapping to Xq26

2. ~ 30 members on the Y chromosome short and long arms. Amplified on the Y. Multiple copies on Y in all eutherian species - ancient on the Y

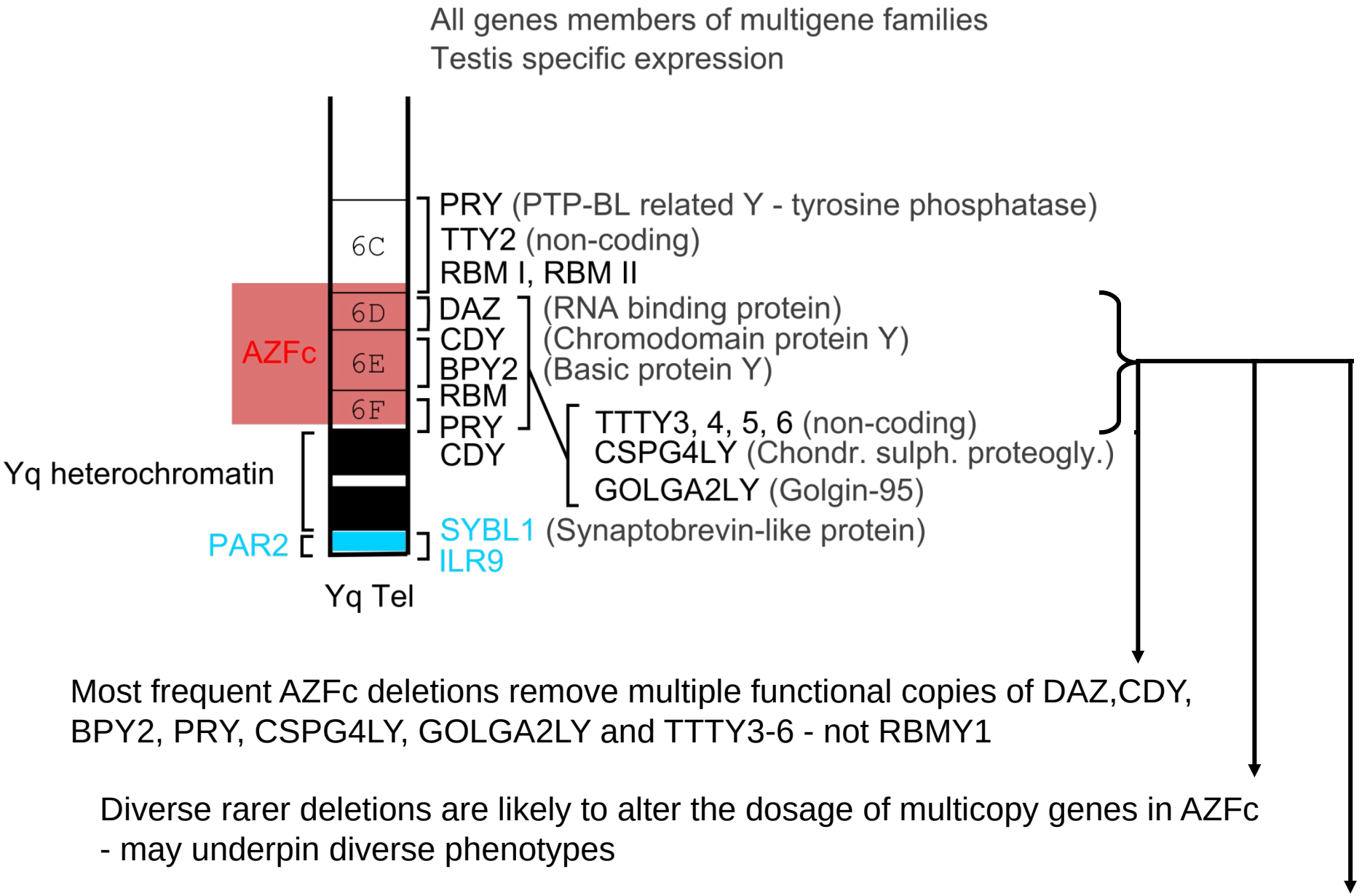
3. Functional copies in AZFb region. AZFb deletions lead to loss of protein expression.

4. Contains 90aa RNA recognition motif that is similar to that found in the autosomal hnRNPG gene; involved in pre-mRNA metabolism.

5. Expressed in nuclei of transcriptionally active germ cells.

6. **However:** in mouse, *Rbmy* is only expressed in later stages of germ cell development, and may not be translated at all. Functional divergence?

AZFc



Most frequent AZFc deletions remove multiple functional copies of DAZ,CDY, BPY2, PRY, CSPG4LY, GOLGA2LY and TTTY3-6 - not RBMY1

Diverse rarer deletions are likely to alter the dosage of multicopy genes in AZFc - may underpin diverse phenotypes

AZFc region is dominated by inverted repeats - instability and phenotype progression

DAZ Gene Family

1. DAZ genes appeared on Y in primates - recent autosomal recruits

2. Multiple transcribed copies of DAZ genes; polymorphic in copy number and order of the DAZ repeats.

3. Transcribed specifically in spermatogonia. Encode proteins with RNA binding motif (like RBM). Potential role in RNA stability.

4. Homologues in other species:
   - *Drosophila Boule* gene
   - Human *DAZLA* (3p24)
   - Mouse *Dazla* (chromosome 17) K.O. no germ cells

Lecture 2: Functional specialisation

- X chromosome
  - *Selective pressures*
  - *Functional categories of X genes*
  - *Disease relevance (human and model systems)*
- Y chromosome
  - *Selective pressures*
  - *Functional categories of Y genes*
  - *Candidate disease genes (Turner syndrome, male fertility)*
- **Structure/function relationships**
  - ***Types of sequence amplification on the sex chromosomes***
  - ***Consequences of repeat structures for pathogenic deletion***
  - ***Transcriptional dynamics of X and Y during spermatogenesis***

Types of sequence amplification involving the sex chromosomes

- Amplification of repeat sequences and “junk DNA”
- Retroposition of Y genes to autosomes
- Y gene amplification with palindrome / inverted repeat formation (moderate copy number, from 2 to a few tens of copies)
- Massive amplification with complex amplicon structure (high copy number, up to several hundred copies)

Types of sequence amplification involving the sex chromosomes

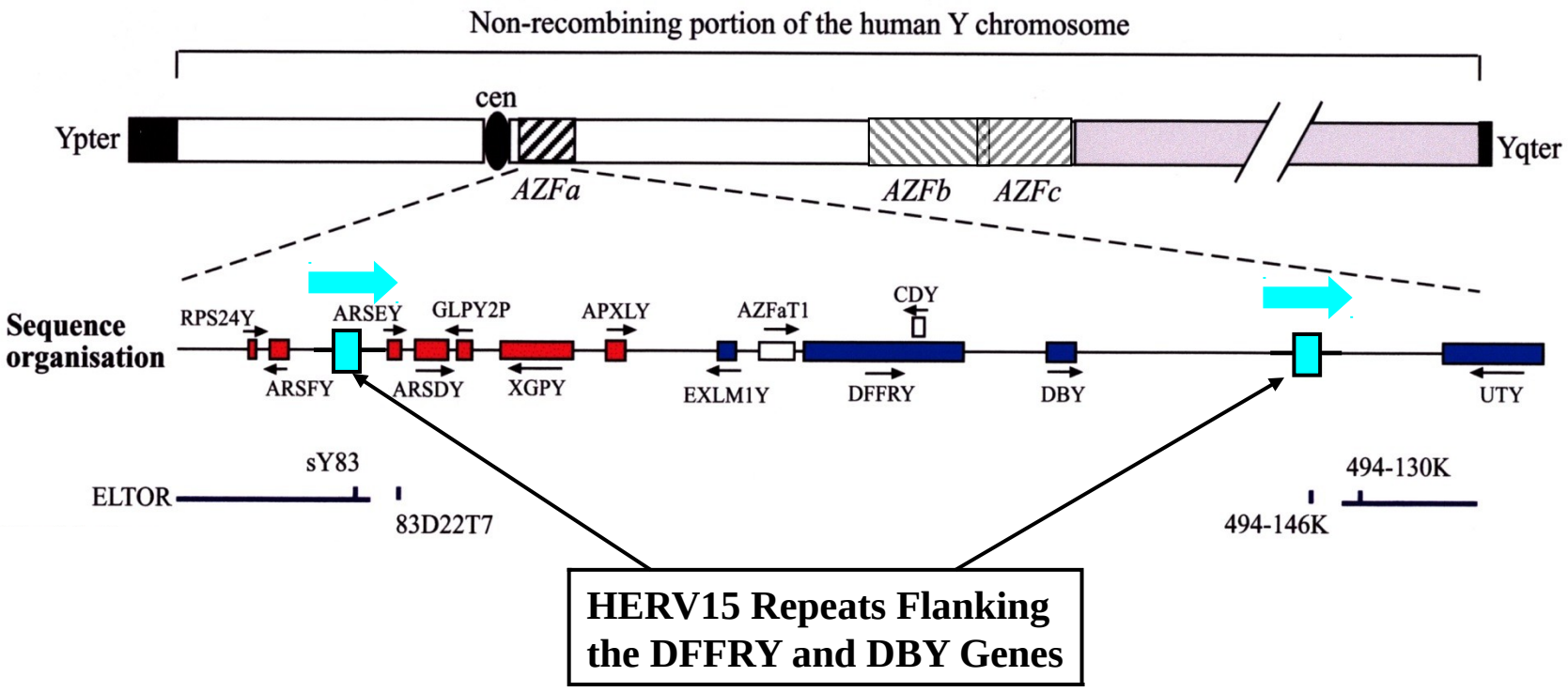
- Amplification of repeat sequences and “junk DNA”
  Part and parcel of the ongoing process of Y chromosome degeneration

- Retroposition of Y genes to autosomes

- Y gene amplification with palindrome / inverted repeat formation
  (moderate copy number, from 2 to a few tens of copies)

- Massive amplification with complex amplicon structure
  (high copy number, up to several hundred copies)

Analysis of AZFa Deletion in Patient ELTOR



Types of sequence amplification involving the sex chromosomes

- Amplification of repeat sequences and “junk DNA”

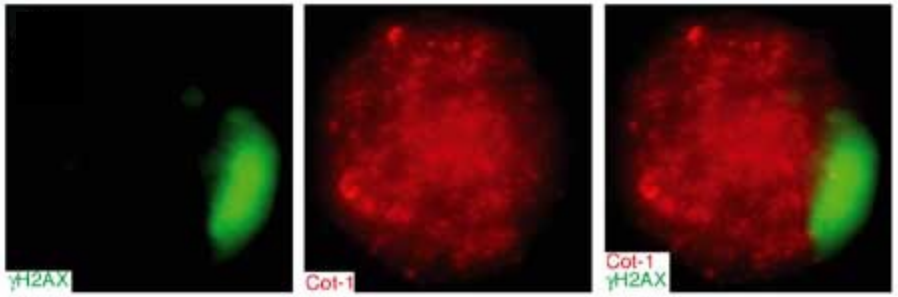
- Retroposition of Y genes to autosomes
  Many X chromosomal housekeeping genes have retroposed copies on autosomes. Retroposed copies tend to be testis specific.
  Explained as a response to MSUC.

- Y gene amplification with palindrome / inverted repeat formation
  (moderate copy number, from 2 to a few tens of copies)

- Massive amplification with complex amplicon structure
  (high copy number, up to several hundred copies)

Meiotic silencing of unpaired chromatin (MSUC)

- Discovered many years ago that the X and Y become transcriptionally inactive during the pachytene stage of meiosis and are sequestered into a repressive facultative heterochromatin domain.



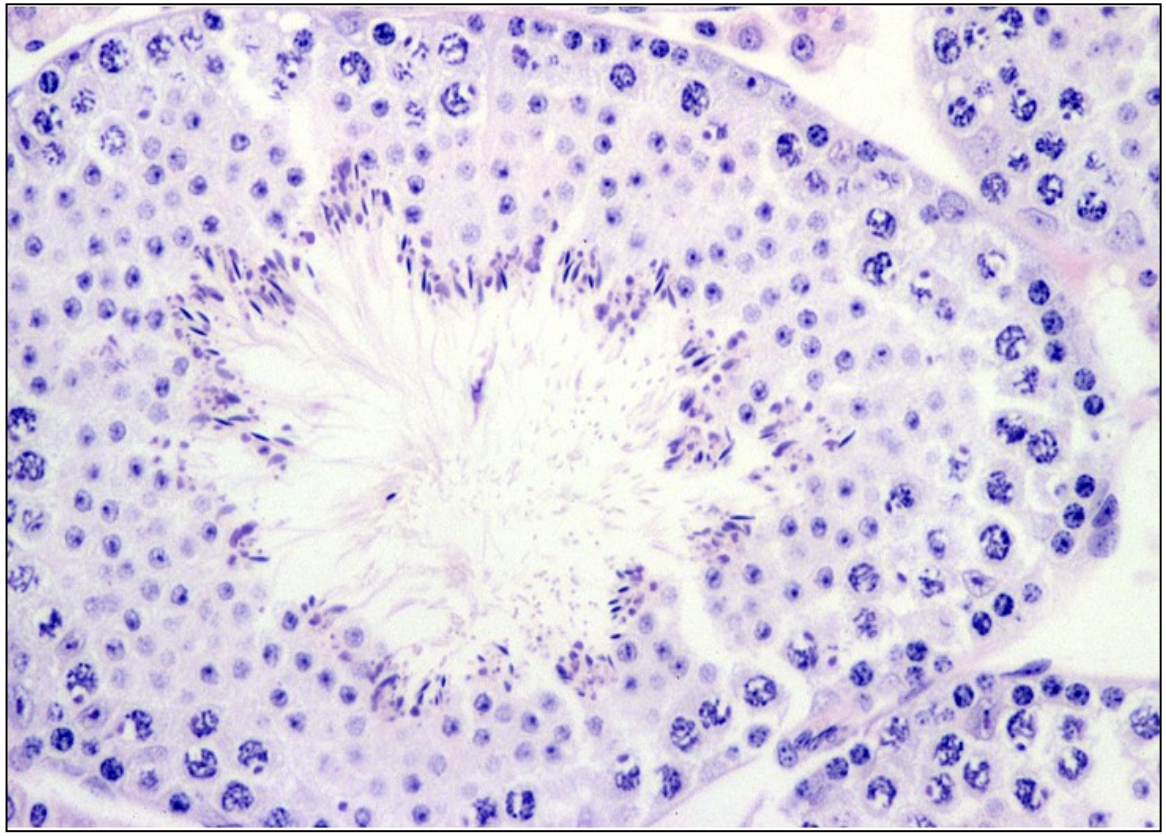
From Turner *et al*
Silencing of unsynapsed meiotic chromosomes in the mouse.

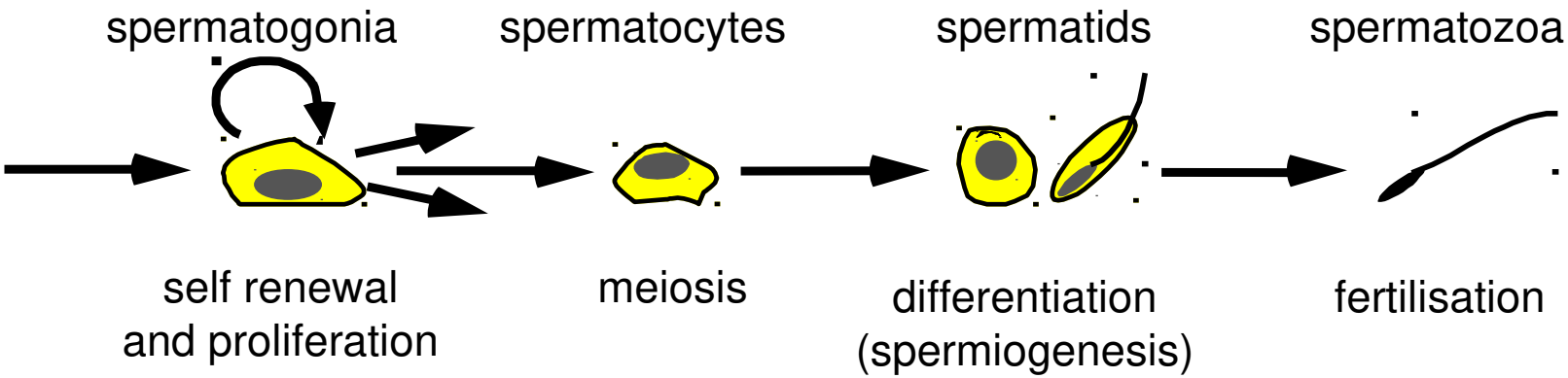
Nature Genetics 37, 41-47

- *Brca1* is recruited to unresolved double strand breaks. Recruits the *Atr* kinase, which phosphorylates the histone H2AX to form γH2AX, which is a marker for silencing. This is a DNA damage repair pathway which has become co-opted for use in meiosis.

- Recently shown to apply to all unsynapsed DNA, not just the sex chromosomes. May be a genomic defence against retroviruses.

- Silencing is maintained into spermatids, with only a few genes reactivating after meiosis is completed




spermatogonia
spermatocytes
spermatids
spermatozoa
self renewal
and proliferation
meiosis
differentiation
(spermiogenesis)
fertilisation
X and Y
active
X and Y
silent
Limited reactivation
of X and Y

Types of sequence amplification involving the sex chromosomes

- Amplification of repeat sequences and “junk DNA”

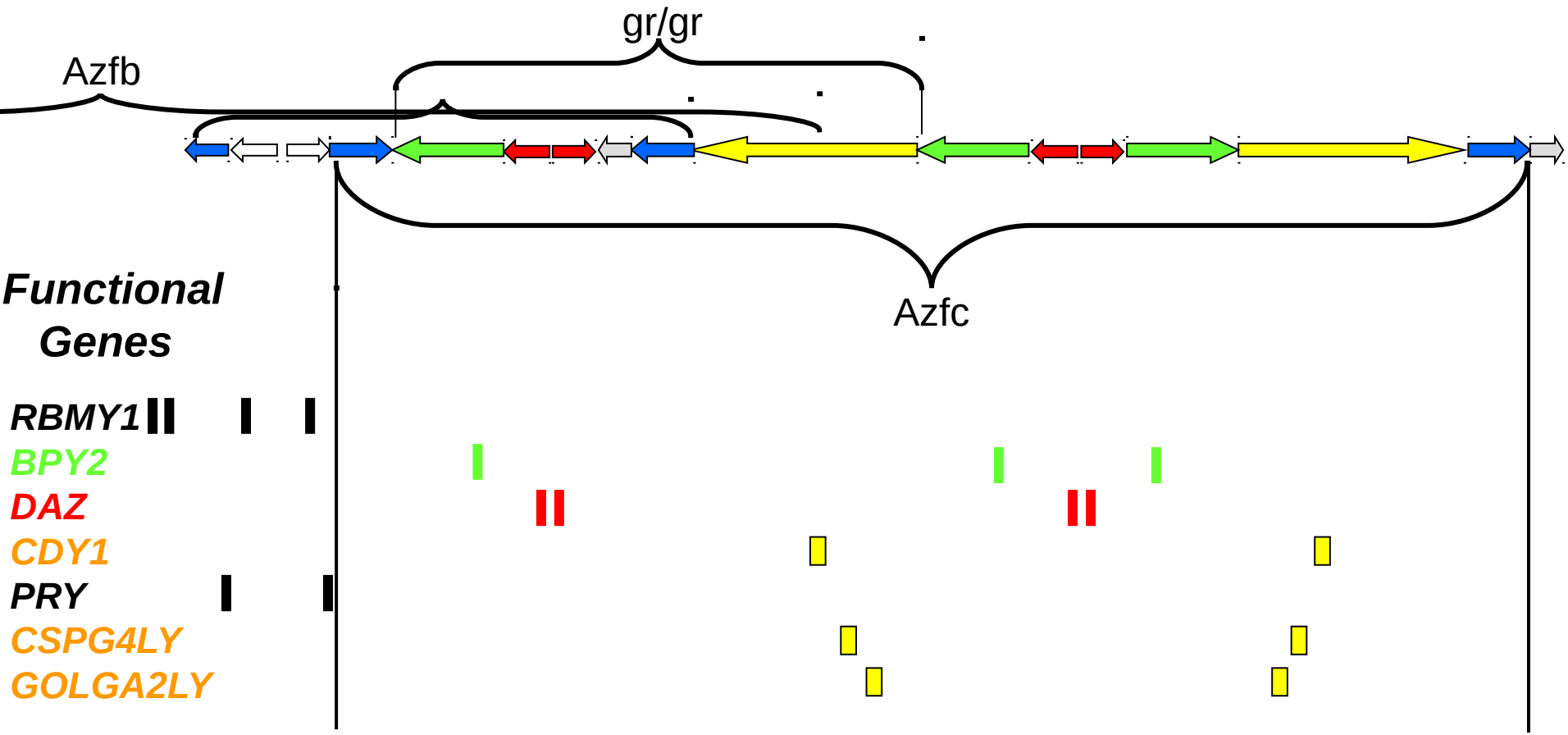
- Retroposition of Y genes to autosomes

- Y gene amplification with palindrome / inverted repeat formation (moderate copy number, from 2 to a few tens of copies)
  Many Y genes are present in multiple copies, arranged in a structured fashion as tandem arrays or palindromes. Large-scale palindromic structures are also found on the X chromosome.

  Less well understood, with several competing explanations

- Massive amplification with complex amplicon structure (high copy number, up to several hundred copies)

Complex Repeat Structure of the AZFc Region



- Rare deletions → copy number variation of AZFc genes - phenotype variation?
- High repeat density → instability - ongoing rearrangement → phenotype progression?
- Inversions (inv. repeats) → varied arrangement of direct repeats - ethnic variants?

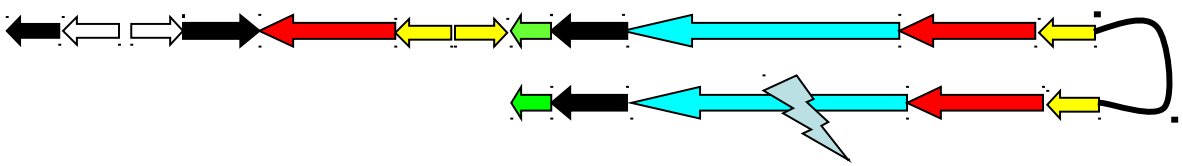
Why are palindromes so prevalent?

Several hypotheses:

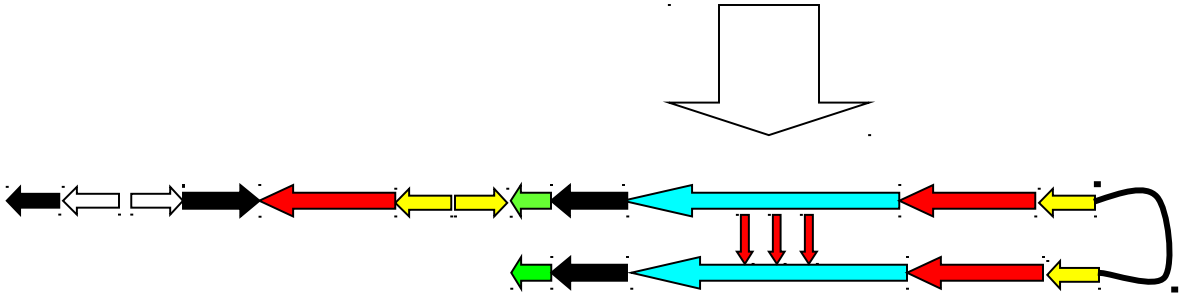
1. It allows for hairpin loop formation and gene conversion, thus allowing the Y to regain some of the benefits of recombination.

Repeat structures may maintain amplified genes by gene conversion

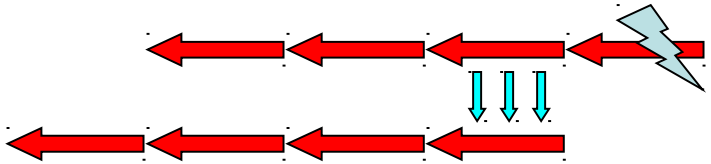
Repeat structures may maintain amplified genes by gene conversion



A single chromatid folds back on itself.

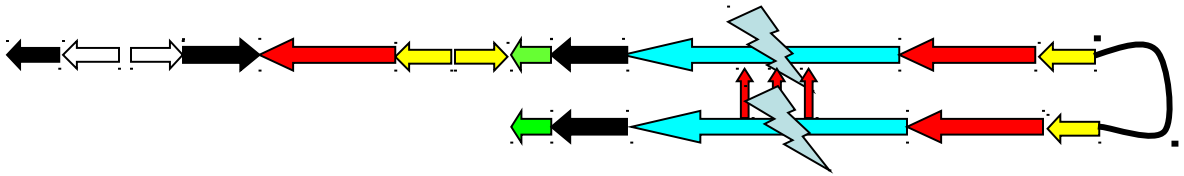


Harmful mutation is repaired by gene conversion from the functional copy

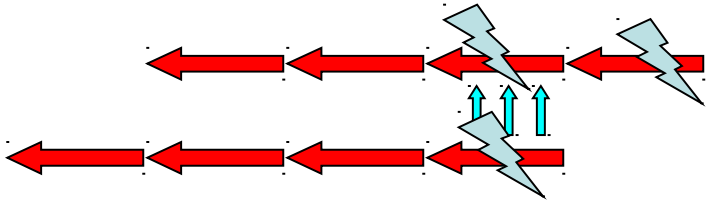


Similarly, sister chromatids can align and repair harmful mutations in copies of a tandem repeat tract

What happens if the conversion runs the other way?



A single chromatid folds back on itself. Harmful mutation is propagated by gene conversion from the mutated copy into the other arm of the IR



Sister chromatids align and amplify a harmful mutation by gene conversion between copies of a tandem repeat tract

Why are palindromes so prevalent?

Several hypotheses:

1. It allows for hairpin loop formation and gene conversion, thus allowing the Y to regain some of the benefits of recombination.

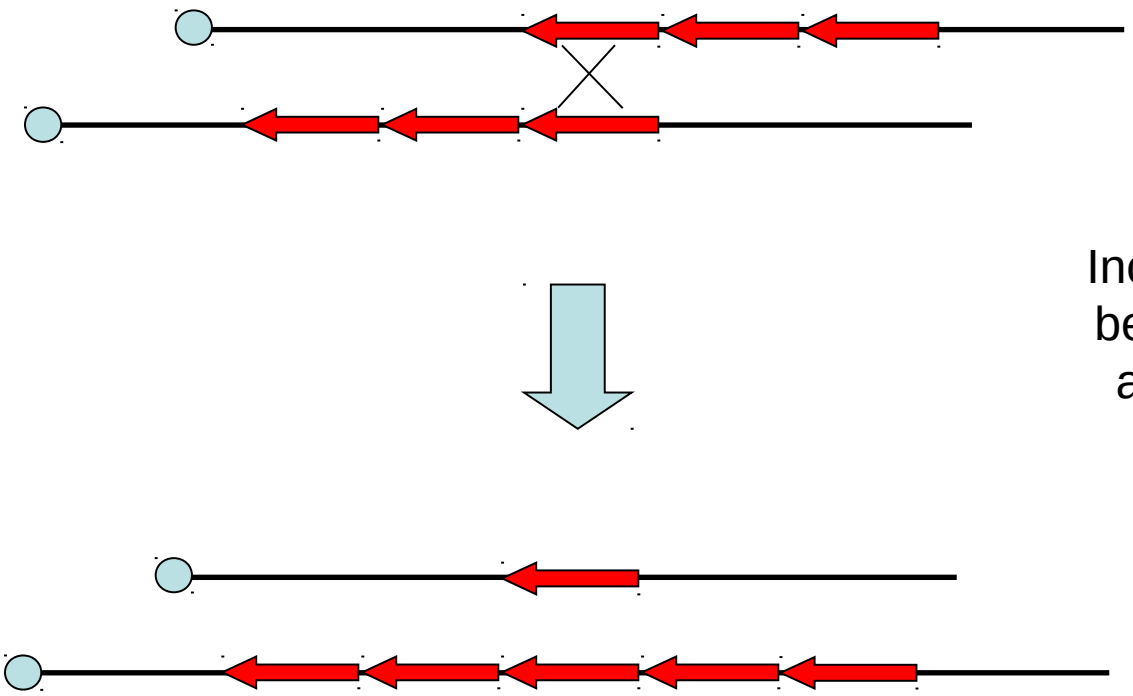
   Problem 1: Gene conversion is not directional. Any given conversion is as likely to eliminate a functional copy as to restore a damaged one. Unclear if there is a net benefit.

   Problem 2: This explanation is Y-centric. The X is also enriched for palindromes, so an argument solely based on the non-recombination of the Y is insufficient.

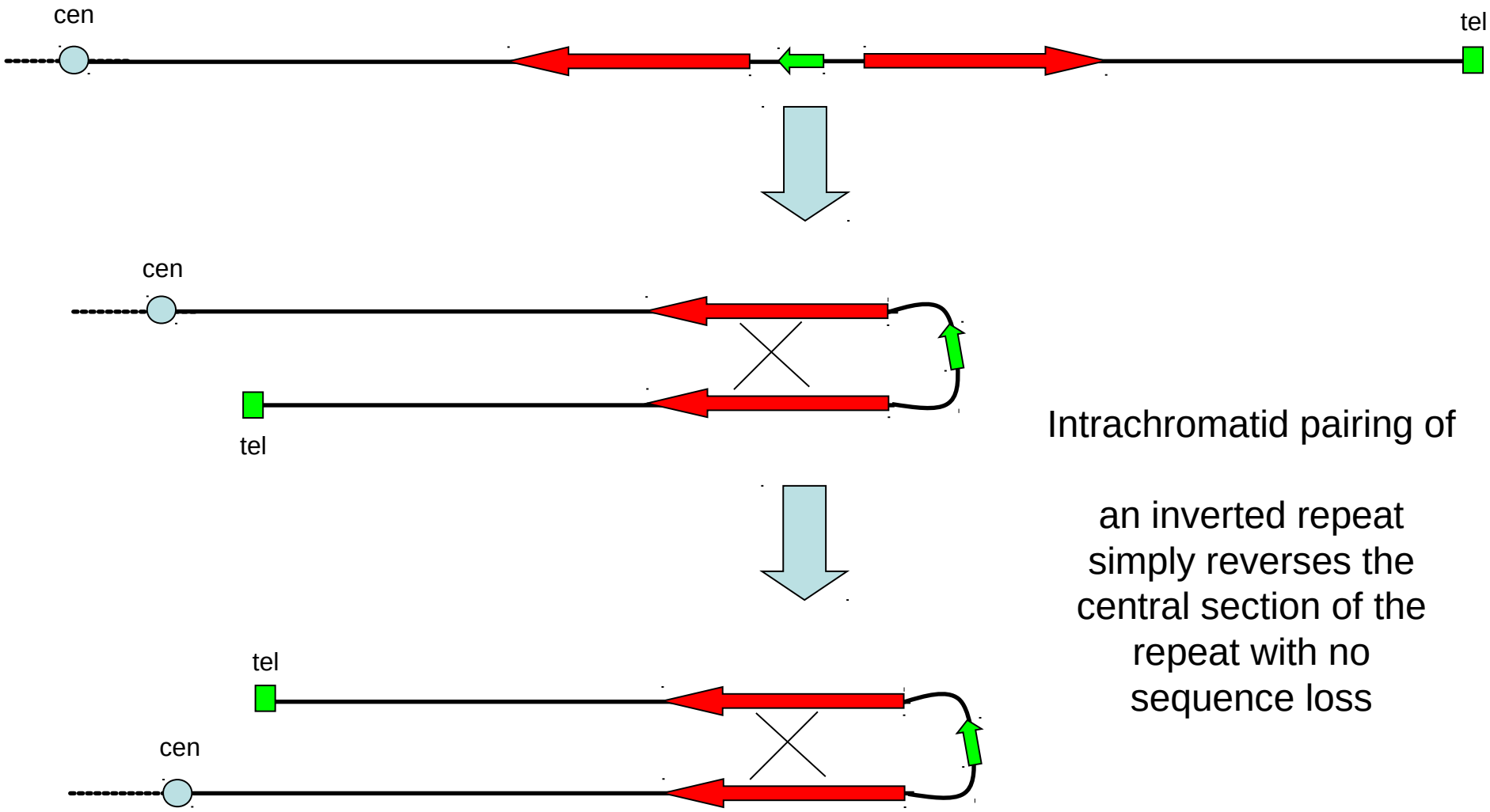
Why are palindromes so prevalent?

Several hypotheses:

1. It allows for hairpin loop formation and gene conversion, thus allowing the Y to regain some of the benefits of recombination.
2. Promotes intra-chromatid pairing. Recombination leads to inversion rather than gene gain or loss. Possibly protective?

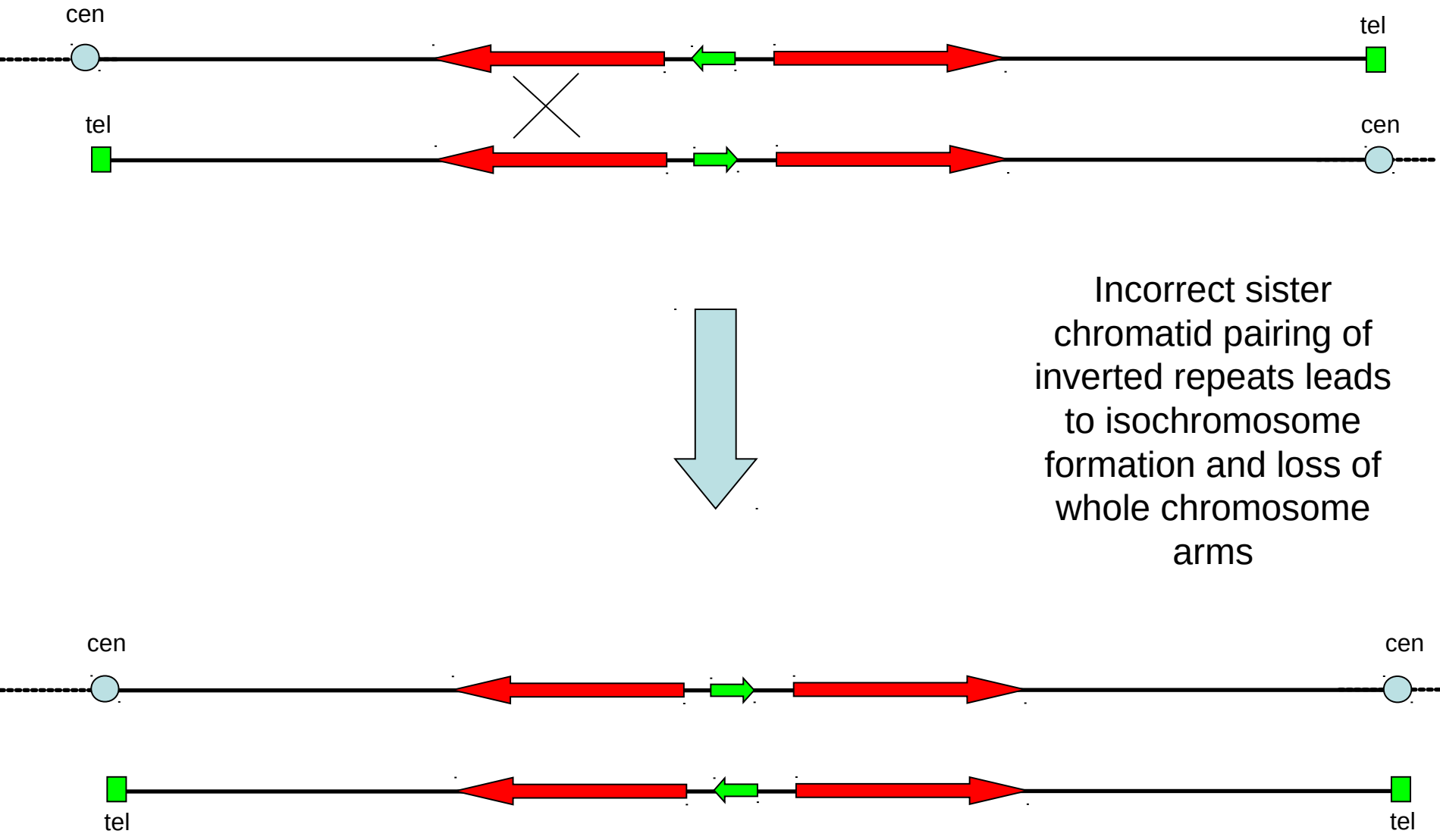


Incorrect recombination between direct repeats always leads to copy number change



Intrachromatid pairing of

an inverted repeat simply reverses the central section of the repeat with no sequence loss



Incorrect sister chromatid pairing of inverted repeats leads to isochromosome formation and loss of whole chromosome arms

For further Y chromosomal examples, see Fig 1 & Fig 4 of the Hughes + Rozen review paper

Why are palindromes so prevalent?

Several hypotheses:

1. It allows for hairpin loop formation and gene conversion, thus allowing the Y to regain some of the benefits of recombination.

2. Promotes hairpin pairing. Recombination leads to inversion rather than gene gain or loss. Possibly protective?

   Problem: increases risk of nonviable isochromosome formation

Why are palindromes so prevalent?

Several hypotheses:

1. It allows for hairpin loop formation and gene conversion, thus allowing the Y to regain some of the benefits of recombination.
2. Promotes intra-chromatid pairing. Recombination leads to inversion rather than gene gain or loss. Possibly protective?
3. Palindromes may have a role in gene expression regulation
   Suggests a reason why genes in palindromes can be overexpressed in tumours – the CT antigen conundrum

Meiotic silencing of unpaired chromatin (MSUC)

MSUC may be a common link explaining two of the unusual features of sex chromosome gene families.

- Autosomal testis-specific retroposons of X-linked housekeeping genes
  Necessary to maintain gene expression when the X is silenced

- Large palindromes on X and Y chromosomes

  Linked to reactivation of X-linked spermatid genes after the period of MSUC. Recent evidence shows that increased copy number on the X and Y is correlated with the degree of spermatid expression of genes

  ? Intra-chromosomal pairing allows evasion / modulation of MSUC?

Types of gene family amplification involving the sex chromosomes

- Retroposition to autosomes
  Many X chromosomal housekeeping genes have retroposed copies on autosomes. Retroposed copies tend to be testis specific.
  Response to MSUC

- Amplification with palindrome / inverted repeat formation
  (moderate copy number, from 2 to a few tens of copies)
  Many testis-specific genes on the sex chromosomes are found in inverted repeat regions. These may become ectopically expressed in cancers – the so-called cancer-testis (CT) antigens. Potential therapeutic targets.
  Preserves sequence via gene conversion and/or a response to MSUC

- Massive amplification with complex amplicon structure
  (high copy number, up to several hundred copies)
  Predominantly found on the mouse X and Y. Human has only a single highly-expanded tract containing the TSPY gene.
  ?

Types of sequence amplification involving the sex chromosomes

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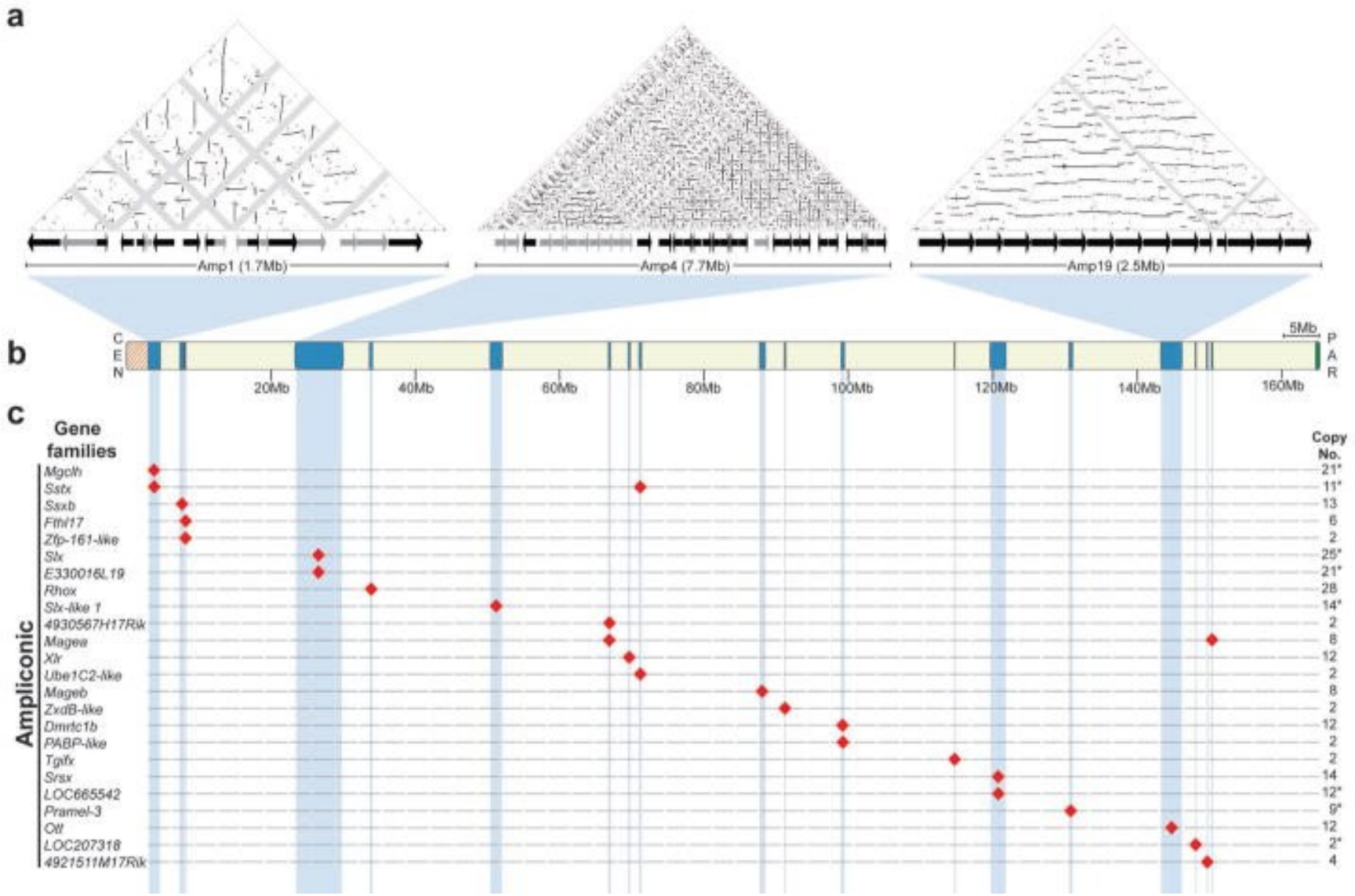
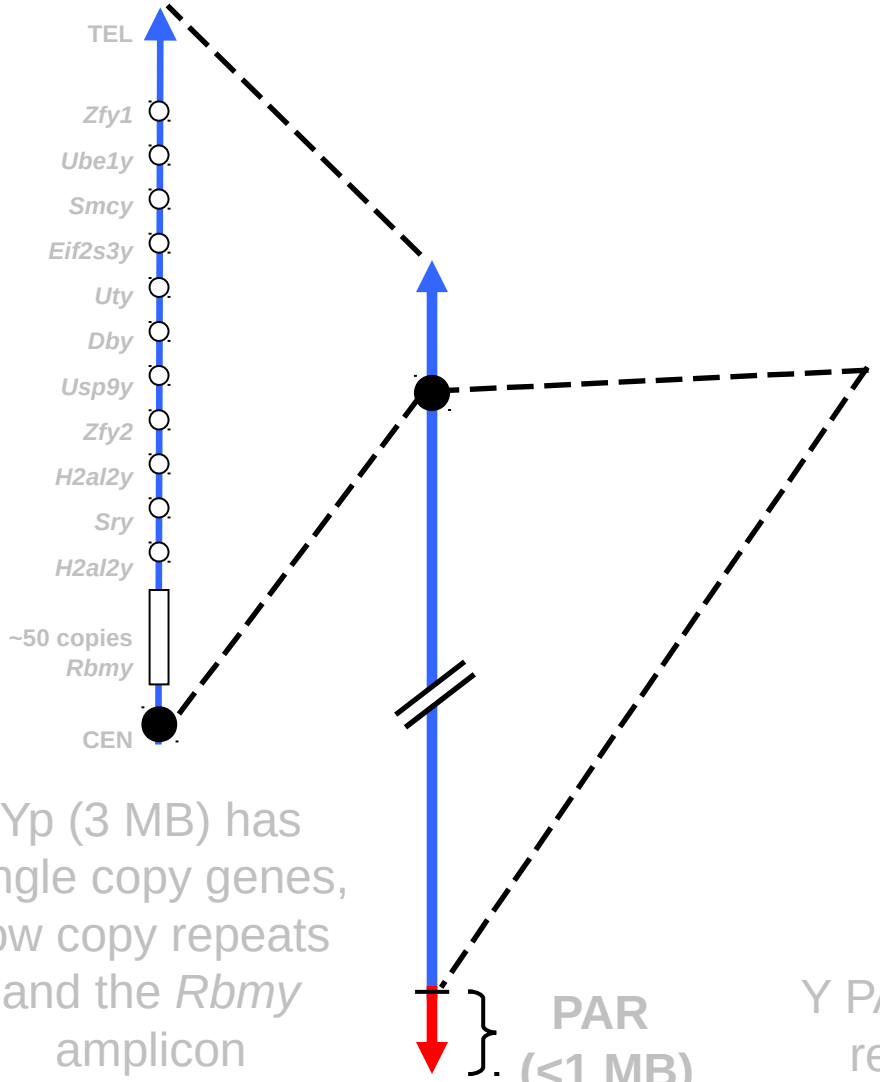


From Mueller *et al*. Nat Genet. 2008 Jun;40(6):794-9.

MOUSE Y CHROMOSOME



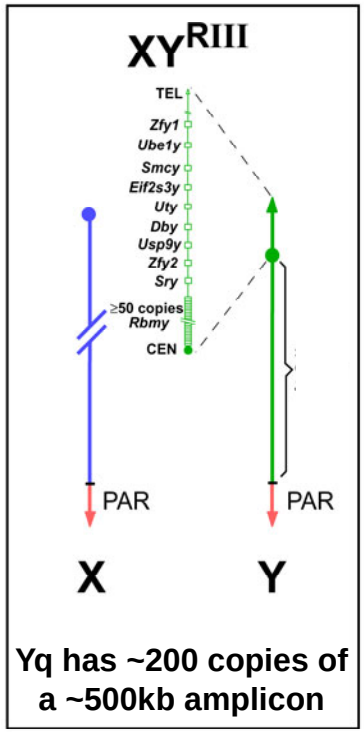
Yq (~92 MB) is massively ampliconic

150-200 copies of a single ~500kb unit called the Huge Repeat Array, interspersed with a few islands of unique sequence. Genes in the HRA present in several hundred copies.

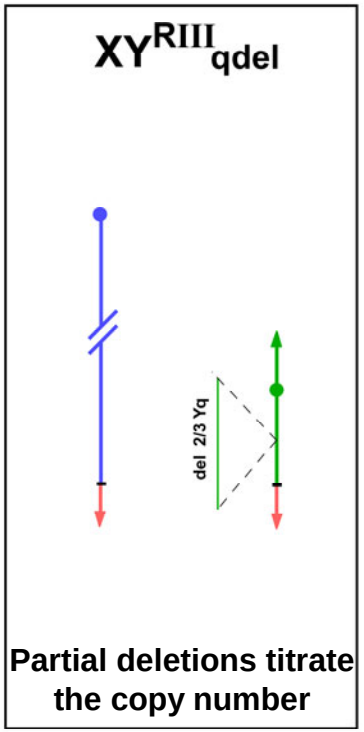
Euchromatin, not heterochromatin!

Yp (3 MB) has single copy genes, low copy repeats and the *Rbmy* amplicon

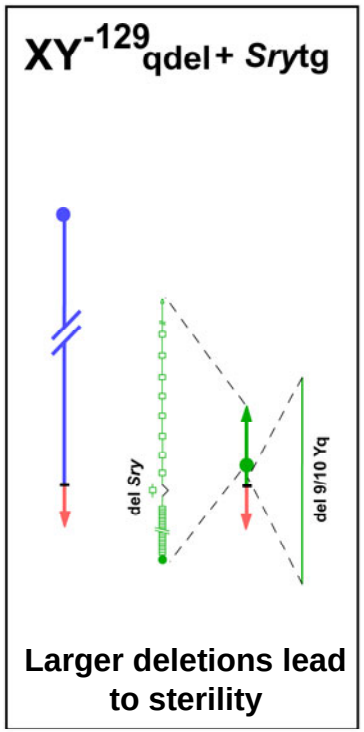
Y PAR pairs (synapses) and recombines with X PAR during male meiosis



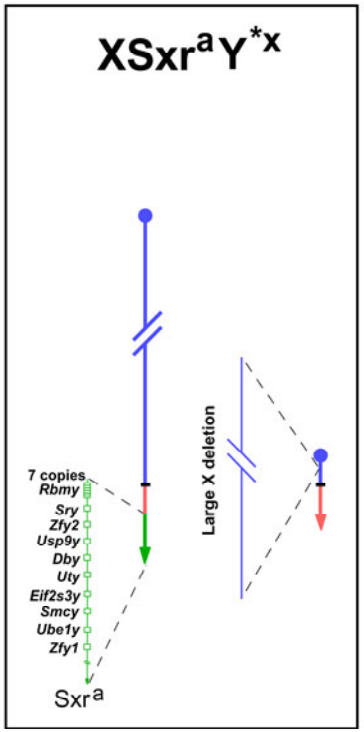
Yq has ~200 copies of a ~500kb amplicon

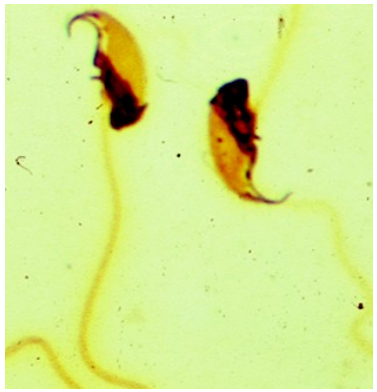


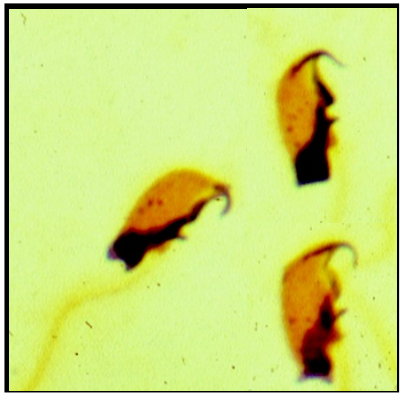
Partial deletions titrate the copy number



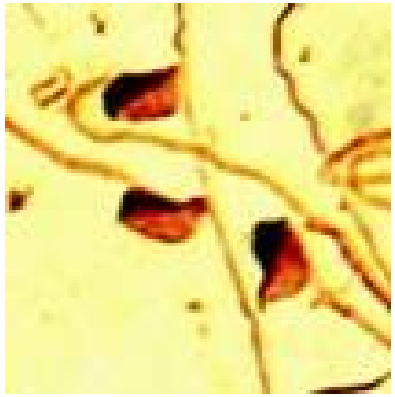
Larger deletions lead to sterility



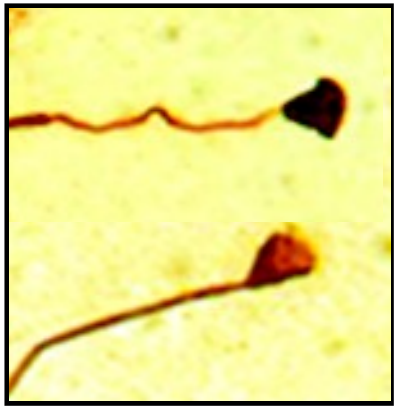




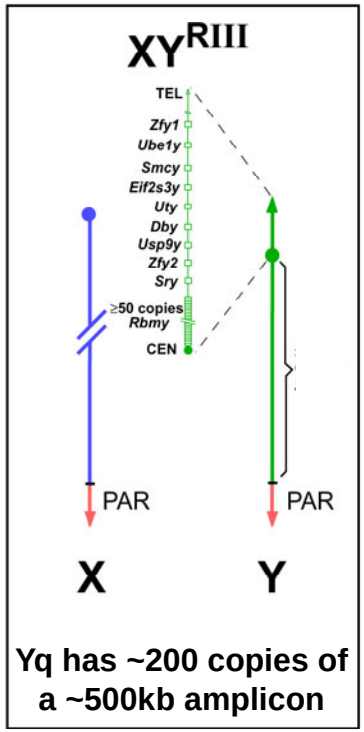
Fertile, **60:40 sex ratio skew in favour of females**



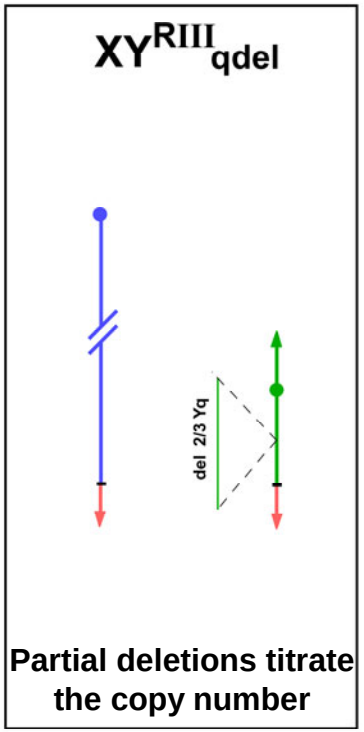
Infertile



Infertile



Yq has ~200 copies of a ~500kb amplicon



Partial deletions titrate the copy number

REDUCED IVF EFFICIENCY

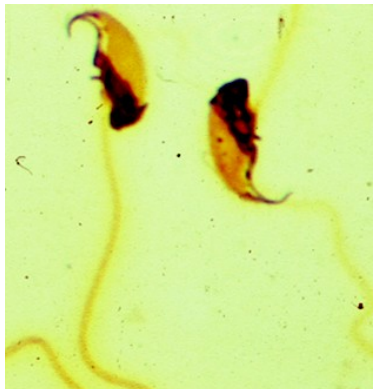
Spermatozoa	No. of eggs	IVF rate
Control	188	79.2 %
2/3 Yq deletion	154	22.1 %*
* Significantly lower than control ($p<0.05$)		

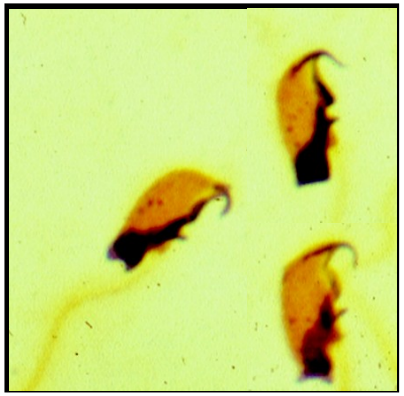
(Xian et al. (1992), **Biol. Reprod.** 47, 549-553)

DISTORTED SEX RATIO

Father	Offspring	Males
Control	187	51 %
2/3 Yq deletion	222	38 %*
* Significantly lower than control ($p<0.05$)		

(Conway et al. (1994), **Mamm. Gen.** 5, 203-210)



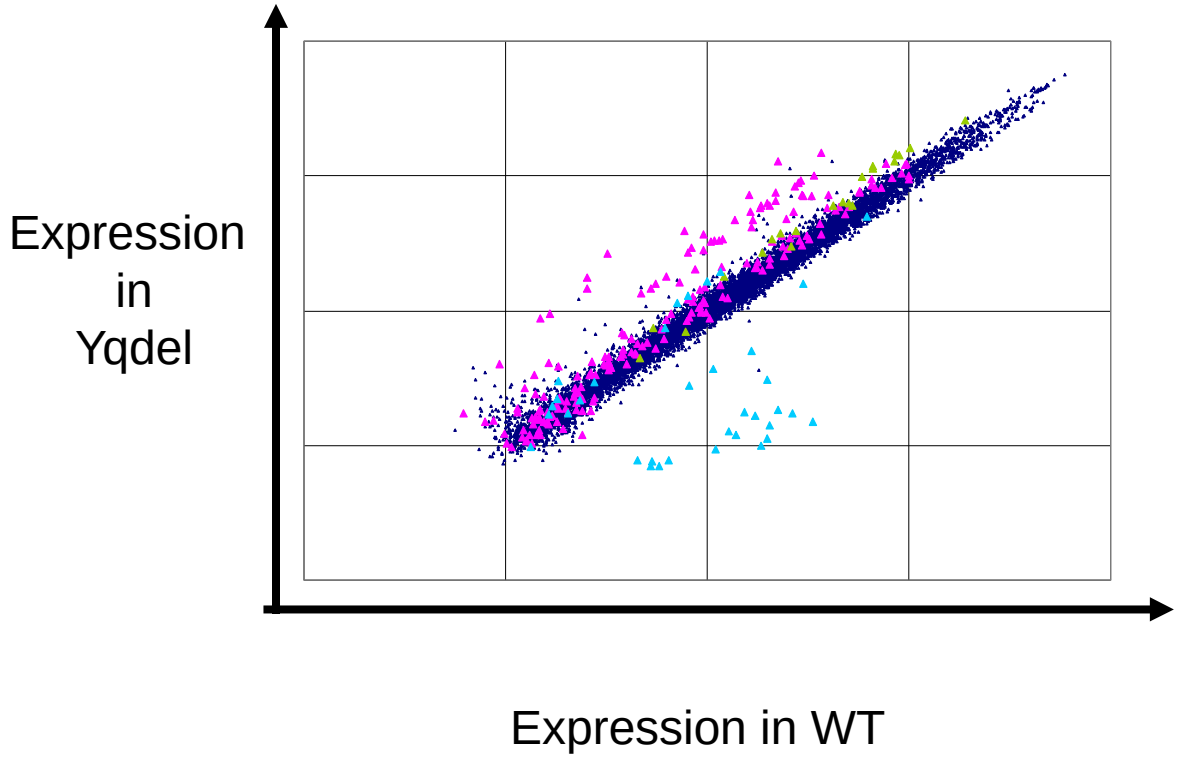


Fertile, **60:40 sex ratio skew in favour of females**

Intracytoplasmic sperm injection (ICSI) abolishes the sex ratio skew.

Conclusion: the defect affects the fertilising ability of Y sperm, not the numbers produced

(Ward et al. (2006) **Biol. Reprod.** 74, 652-658)



Deletions on mouse Yq lead to widespread overexpression of X linked genes, associated with offspring sex ratio distortion

Types of gene family amplification involving the sex chromosomes

- Amplification of repeat sequences and “junk DNA”
  Part and parcel of the ongoing process of Y chromosome degeneration
- Retroposition of Y genes to autosomes
  Many X chromosomal housekeeping genes have retroposed copies on autosomes. Retroposed copies tend to be testis specific.
  Response to MSUC
- Amplification with palindrome / inverted repeat formation
  (moderate copy number, from 2 to a few tens of copies)
  Many testis-specific genes on the sex chromosomes are found in inverted repeat regions. These may become ectopically expressed in cancers – the so-called cancer-testis (CT) antigens. Potential therapeutic targets.
  Preserves sequence via gene conversion and/or a response to MSUC
- Massive amplification with complex amplicon structure
  (high copy number, up to several hundred copies)
  Predominantly found on the mouse X and Y. Human has only a single highly-expanded tract containing the TSPY gene.
  Genomic conflict over offspring sex ratio in mouse. Other species (?)

REFERENCES – Function

1 Genomics and Genetics of Human and Primate Y Chromosomes.
Hughes JF, Rozen S. **Annu Rev Genomics Hum Genet.** 2012 Apr 5.
http://www.annualreviews.org/doi/pdf/10.1146/annurev-genom-090711-163855
Comprehensive coverage of human Y evolution.

2 Inverted repeat structure of the human genome: the X-chromosome contains a preponderance of large, highly homologous inverted repeats that contain testes genes.
Warburton PE, Giordano J, Cheung F, Gelfand Y, Benson G. **Genome Res.** 2004 Oct;14(10A):1861-9.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC524409/
Identification of large scale palindromic / inverted repeats on X and Y in human and mouse, and the relation to testis gene expression.

3 Genes that escape from X inactivation.
Berletch JB, Yang F, Xu J, Carrel L, Disteche CM. ***Hum Genet*.** 2011 Aug;130(2):237-45.
http://www.springerlink.com/content/l40271075w372267/fulltext.pdf
A mechanistic look at genes escaping X inactivation and clinical perspective on related phenotypes

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(Extra reading for those interested in the genomic conflict story in mouse)

Deletions on mouse Yq lead to upregulation of multiple X- and Y-linked transcripts in spermatids.
Ellis PJ et al. ***Hum Mol Genet****. 2005 Sep 15;14(18):2705-15.*

A genetic basis for a postmeiotic X versus Y chromosome intragenomic conflict in the mouse.
Cocquet J et al. ***PLoS Genet.*** *2012 Sep;8(9):e1002900.*

Genomic conflict hypothesis

- An X-linked gene or genes acts to distort the offspring sex ratio in favour of females

- A Y-linked gene or genes acts to repress the activity of the X-linked distorters, restoring a normal offspring sex ratio

- This leads to an “arms race” between competing gene families on both X and Y chromosomes. Increasing copy number on one sex chromosome drives increasing copy number of the other, and *vice versa*

- Partial deletion of the “repressor” gene complexes on the Y chromosome relieves the X gene inhibition and uncovers the sex ratio phenotype

Mouse Yq

Hundreds of copies of six genes, all in a single amplicon of ~500kb called the Huge Repeat Array which is repeated ~200x on mouse Yq (~100MB).

- *Ssty1 / Ssty2*
  Two related families of spindlin-like genes.

- *Sly*
  A COR1 domain protein (potentially chromatin-binding).

- *Asty*, *Srsy*, *Orly*
  Less well-characterised transcripts. Unclear if any protein is produced, though *Srsy* at least is predicted to encode a fair-sized ORF.

- *Orly*
  A chimeric locus composed of partial copies of *Ssty1*, *Asty* and *Sly.* It is transcribed in both directions. Potential for RNAi inhibition of related genes.

Mouse Yq

Hundreds of copies of six genes, all in a single amplicon of ~515kb called the Huge Repeat Array which is repeated ~200x on mouse Yq (~100MB).

- *Ssty1 / Ssty2*
  Two related families of spindlin-like genes.

- ***Sly***
  A COR1 domain protein (potentially chromatin-binding).
  Yeast 2-hybrid data showing interaction with histone modification proteins

- *Asty*, *Srsy*
  Less well-characterised transcripts. Unclear if any protein is produced, though *Srsy* at least is predicted to encode a fair-sized ORF.

- ***Orly***
  A chimeric locus composed of partial copies of *Ssty1*, *Asty* and *Sly*. It is transcribed in both directions. Potential for RNAi inhibition of related genes.

Sly knockdown recapitulates the sperm head deformities seen in MSYq deletion

Sex ratio data:

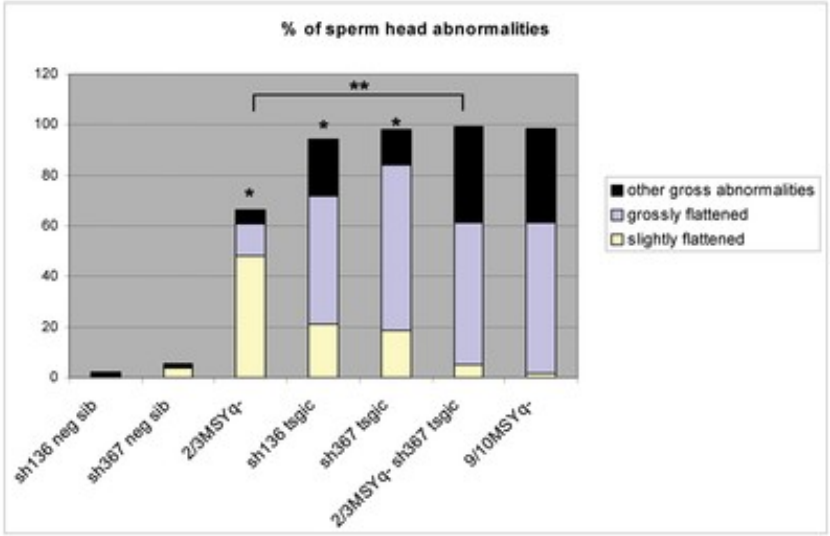
Control WT: 176 / 368 = 47.8% female

shSLY: 55 / 96 = 57.3% female

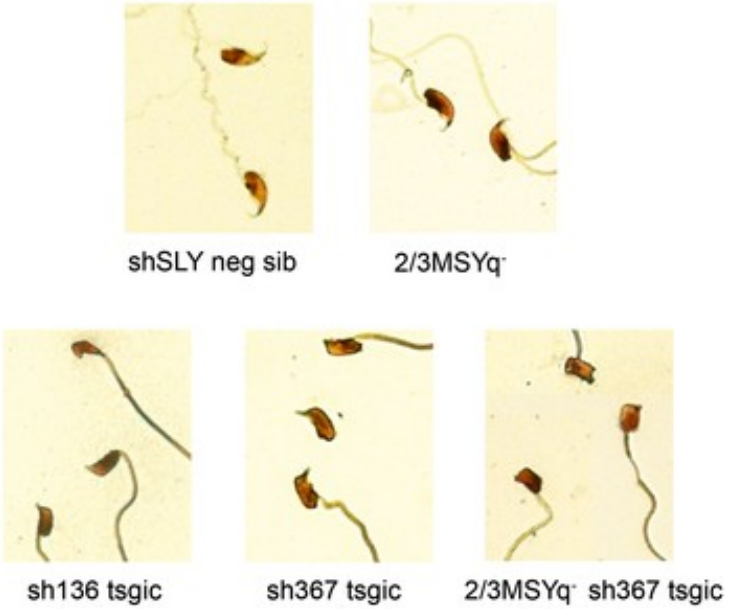
p value = **0.049*** (single tailed test)

… and the sex ratio skew too, although the significance is marginal

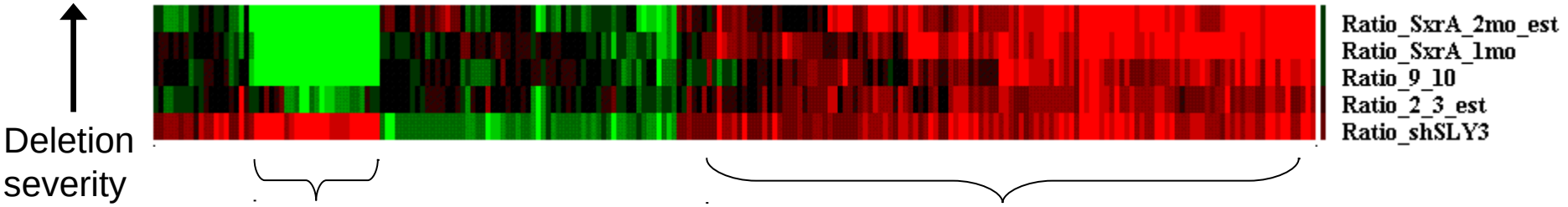
A



B



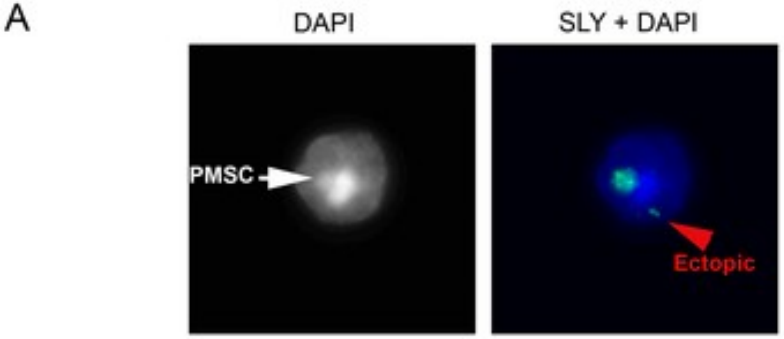
Investigating *Sly* – transcriptional data

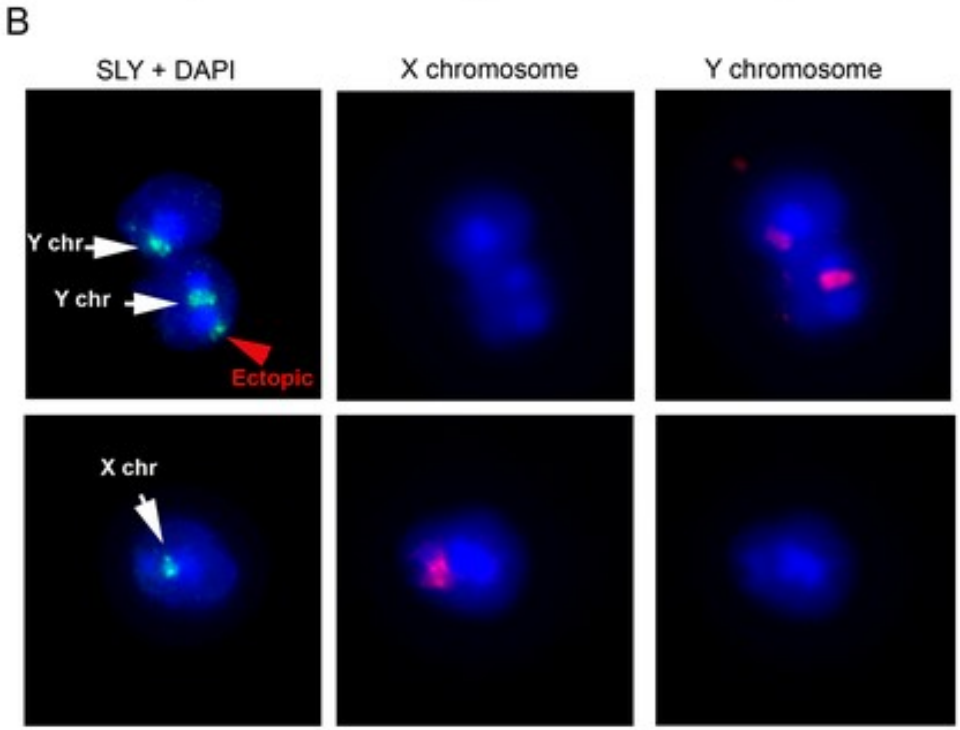


Other (non-*Sly*) Y-linked genes are upregulated in response to the knockdown

shSLY knockdown of *Sly* transcript levels recapitulates the gross X upregulation seen in MSYq deleted spermatids.

Sly is a global regulator of sex chromosome transcription in spermatids. This is likely to relate to the re-activation of sex chromosome transcription in spermatids following meiotic silencing





C

	% of spermatids
PMSC colocalisation (+/- ectopic)	66% (136/206)
Outside	2.5% (5/206)
No signal	31.5% (65/206)

Spermatid with PMSC signal	% of spermatids
Y-bearing	56% (47/84)
X-bearing	44% (37/84)

Sly protein localises to the sex chromosomes in spermatids

i.e. it is a plausible chromatin regulator affecting global X/Y postmeiotic expression

Recently shown that the X homologues *Slx/Slxl1* also localise to the sex chromosomes and have the opposite effect on both gene expression and sex ratio – i.e. a true "selfish" genomic conflict

(Cocquet et al. (2009) **PLoS Biol.** 7(11):e1000244)

Unravelling the genomic conflict

- *Sly* on the Y chromosome and *Slx / Slxl1* on the X chromosome are conclusively shown to be involved in a genomic conflict driving amplification of gene copy number on both chromosomes.

- They localise to sex chromosomes in spermatids and appear to act by modulation of post-meiotic silencing (MSUC).

- These are however upstream regulatory effects. Identification of the downstream X-linked targets that actually mediate the skew may potentially allow for targeted modification of sex ratio in commercial species (cow, pig etc.)

- The conflict appears to be an important contributory factor to speciation in mouse – could similar X/Y dynamics be more generally important in reproductive isolation in other species?
(*Haldane's rule: sterility in intraspecific hybrids predominantly affects the heterogametic sex*)